Published in final edited form as:

Anal Methods. 2016 October 7; 8(37): 6696–6700. doi:10.1039/C6AY02102F.

Multiplexed detection of pathogen-specific DNA using engineered zinc finger proteins without target amplification

Moon-Soo Kim^{a,*} and Juhwa Kim^a

^aDepartment of Chemistry, Western Kentucky University, Bowling Green, KY 42101 USA

Abstract

Multiplexed detection of pathogen-specific DNA sequences in a simple and reliable way is in great demand for clinical and biomedical applications. However, there is still a lack of available DNA detection methods that are simple and pathogen-selective for point-of-care (POC) testing. Here, we report a novel zinc finger protein (ZFP)-based chemiluminescent method for direct detection of pathogenic double-stranded DNA (dsDNA) in a multiplexed platform. ZFPs are custom-designed to identify unique pathogenic DNA sequences. ZFP-based chemiluminescent detection of dsDNA provides sufficient sensitivity (50 fmol) and high specificity without target DNA amplification. Our study addresses the potential of developing a simple and selective pathogen detection method in a multiplexed fashion needed for POC application.

The development of a new technology for detection of double-stranded (ds) DNA sequences enables diverse diagnostic applications such as identifying multiple pathogens simultaneously. The availability of an inexpensive and simple technology for detection of specific pathogenic bacteria will allow for effective treatment, improving healthcare of millions of people. The conventional microbiological detection of pathogenic bacteria relies on culture methodology and requires days to yield results.¹² As an alternative to the culture-based method, nucleic acid amplification based methods have been offered for a fast and sensitive detection of bacterial genome.^{3–6} However, detection based on nucleic acid amplification requires dsDNA denaturation and subsequent renaturation with specific primers or probes under controlled conditions, and analysis of internal control sequences of PCR (polymerase chain reaction) products.^{7, 8} Therefore, direct detection of dsDNA allows us to avoid the need for denaturation and subsequent hybridization and a complicated detection procedure by PCR.

A methodology for direct reading of the sequence information of dsDNA has been developed through interactions with DNA-binding zinc finger proteins (ZFPs).^{9–13} The Cys2-His2 zinc finger (ZF) is one of the most common DNA-binding domains. Zinc ion is tetrahedrally coordinated between two cysteine and two histidine residues.¹⁴ Each ZF forms a $\beta\beta\alpha$ structure, stabilized by hydrophobic interactions and zinc coordination.¹⁴ A ZF domain recognizes three to four nucleotides of DNA and can be assembled into a tandem array to form multi-finger domains using a modular assembly approach.¹⁵ For instance, six

moon-soo.kim@wku.edu; Fax: +1 270 745 5361; Tel: +1 270 745 4362.

Electronic Supplementary Information (ESI) available: Target DNA sequences, EMSA. See DOI: 10.1039/x0xx00000x

ZF domains can recognize 18 base pairs of a desired DNA sequence, which would be sufficient enough to specify a unique site within the all known genome.^{16–18} Since the human genome is much bigger than the bacterial genome, a shorter DNA sequence may be required to specify a single site. For pathogenic bacteria, a 12 bp of DNA sequence would occur once in 16.77×10^6 nucleotides, which is bigger than the sizes of an *E. coli* O157 genome (5.44×10^6) and a *Staphylococcus aureus* genome (2.82×10^6). Therefore, in principle, at least four ZF domains may be required to confer specificity for detecting bacterial pathogenic DNA. The binding specificity of ZFs is determined by the α -helix region, which is inserted into the major groove of DNA. Amino acid residues at positions -1, 3, and 6 (relative to the start of α -helix) in the α -helix make specific contacts to three bases.¹⁹

Previously, a colorimetric detection method to achieve visual signal of DNA detection have been demonstrated.^{9, 10} In this method, sequence-enabled reassembly of β -lactamase (SEER-LAC) system⁹ was applied for enzymatic amplification of a reporter enzyme TEM-1 β -lactamase, which hydrolyzes its substrate, generating color change from yellow to red. In the SEER-LAC system⁹, 29 kDa β -lactamase was dissected into two inactive fragments and each split enzymatic domain was fused with a ZFP in each ZFP pair. This assay was performed both in solution⁹ and on a solid surface coated with poly (ethylene glycol)(PEG) hydrogel.¹⁰ We previously developed a ZFP array with the SEER-LAC system to detect a bacterial dsDNA sequence to achieve a point-of-care (POC) system using ZFPs.¹⁰ The SEER-based method with a different reporter molecule reassembly has been investigated using luciferase and a green fluorescence protein (GFP).^{13, 20} The correct reassembly of the reporter molecule from split-peptide fragments indicated dependence on the recovery of the structural geometry inherent to the reporter protein.²¹

The enzyme-based signal amplification method using chemiluminescence has been widely used for diagnostic applications such as immunoassays and reporter assays.^{22–25} Among the numerous assays for detecting antigen and nucleic acids, the chemiluminescent enzyme immunoassay shows both high sensitivity and specificity. It has been applied broadly in clinical and biomedical research due to such advantages as relatively simple and inexpensive instrumentation, low detection limit, and broad dynamic range.^{23–26} Chemiluminescent detection methods commonly employ horseradish peroxidase (HRP) and alkaline phosphatase (ALP) for enzymatic signal amplification.

In this study, we developed a multiplexed chemiluminescent detection method for pathogen-specific dsDNA using engineered ZFPs with no enzymatic domain attached. Our approach shows the direct detection of multiple dsDNA sequences present in different pathogenic bacteria in a single assay, which circumvents the need for DNA denaturation and hybridization. Biotin is simply conjugated to a detection probe ZFP via lysine moieties of a maltose binding protein (MBP) fused to the ZFP and a capture ZFP probe is immobilized on nitrocellulose membrane. The sequence-specific detection of target pathogenic dsDNA by ZFPs is transduced into chemiluminescent signal using the streptavidin-HRP assay.

Experimental

Materials and solutions

Bio-dot microfiltration apparatus, nitrocellulose membrane, and Bio-dot SF filter papers were purchased from Biorad (Hercules, CA). Streptavidin-Horseradish Peroxidase (SA-HRP) conjugate, Sulfosuccinimidyl-4-[Nmaleimidomethyl] cyclohexane-1 carboxylate (sulfo-SMCC), N-succinimidyl-S acetylthio- propionate (SATP), EZ-link sulfo-NHS-LC-LC-biotin, and Supersignal West pico chemiluminescent substrates were obtained from Thermo Scientific. The target DNA oligonucleotide was obtained from IDT. Tris buffer containing Zn^{2+} (TZ buffer, pH 8.5) consisted of 100 mM tris, 90 mM KCl, 0.1 mM ZnCl_2 , and 1 mM MgCl_2 . HEPES buffer (pH 7.5) contained 100 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 90 mM KCl, 0.1 mM ZnCl_2 , and 1 mM MgCl_2 . Zinc Buffer A (ZBA) was made of 10 mM Tris, pH 7.5, 90 mM KCl, 1 mM MgCl_2 , and 90 μM ZnCl_2 . PBST/ Zn^{2+} buffer consisted of 100 mM phosphate, 150 mM NaCl, 0.05 % Tween-20, and 90 μM ZnSO_4 .

Construction, expression and purification of ZFPs

Two pairs of ZFPs were engineered to recognize a pathogenic strain of STEC (Shiga toxin-producing *E. coli*) and *Staphylococcus aureus*. Each ZFP was constructed by the modular assembly method using the Barbas set of modules in a modified Sp1C framework²⁷. The DNA coding region for each ZFP was commercially synthesized by Bio Basic. ZFPs were subcloned between the BamHI and HindIII sites of pMAL-c2X full-length β -lactamase, replacing a full-length β -lactamase. The vector enables bacterial expression of the proteins as fusions with an N-terminal maltose binding protein (MBP) as a purification tag. Proteins were expressed in *E. coli* BL21 (Invitrogen) upon induction with 0.3 mM Isopropyl b-D-1-thiogalactopyranoside (IPTG) at an OD600 of 0.6–0.8 for 3 h at 37 °C. Cells were pelleted and resuspended in Zinc Buffer A (ZBA) including 5 mM dithiothreitol (DTT) and 50 mg ml^{-1} RNase A. After sonication, proteins in cell lysate were applied to an amylose resin column pre-equilibrated with ZBA containing 5 mM DTT, washed with ZBA containing 2 M NaCl and ZBA containing 1 mM tris(2-carboxyethyl) phosphine (TCEP), and eluted in ZBA containing 10 mM maltose and 1 mM TCEP. Concentration and purity were assessed by Coomassie-stained polyacrylamide gel electrophoresis with sodium dodecyl sulfate (SDS-PAGE) using bovine serum albumin (NEB) standards. A purified protein was stored in ZBA containing 1 mM TCEP at 4 °C until use.

Biotin conjugation

The biotin-conjugated ZFP was prepared by cross-linking EZ-link sulfo-NHS-LC-LC-biotin and the amine group of a MBP of the capture probe ZFP.¹¹ Zn^{2+} -containing HEPES buffer was used to maintain the ZFP structure during chemical conjugation.¹¹ 500 μL of HEPES buffer containing 200 $\mu\text{g mL}^{-1}$ ZFP was mixed with 10 μL of 4 mM EZ-link sulfo-NHS-LC-LC-biotin and incubated for 2 h at 4 °C, and the mixture was then filtered by centrifugation for 20 min. Afterward, the filtrate was dissolved in 1 mL of TZ buffer.

Multiple target DNA detection using engineered ZFPs

The sequences of the single-stranded DNA (ssDNA) oligonucleotide used to form target dsDNA are provided in the Supplementary Information. The dsDNA target oligonucleotide was prepared by heating 250 μ M ssDNA at 95°C for 10 min, then slowly cooling to room temperature to form hairpins containing a four-thymidine loop. For further use, DNA was diluted in PBSTM (0.1% milk (w/v) in PBST/ Zn^{2+} buffer). The engineered ZFP capture probe was immobilized on the nitrocellulose membrane by using a Bio-dot microfiltration apparatus according to the manufacturer's instruction. The membrane was coated with different concentrations (100 nM, 150 nM, 200 nM, 400 nM, 800 nM, 1.6 μ M) of ZFP SEB400 and Stx2-268 in different wells of the chamber. Next, the ZFP-coated membrane was washed twice with PBST/ Zn^{2+} buffer under vacuum, followed by blocking with 1% BSA in PBST/ Zn^{2+} by gravity flow in the microfiltration apparatus. Target DNA oligonucleotides were applied onto ZFP-coated membrane by gravity flow. After washing the membrane twice with PBST/ Zn^{2+} , each biotinylated ZFP (SEB435 and Stx2-233) was applied to each well by gravity flow. The membrane was subsequently washed twice with PBST/ Zn^{2+} and treated with SA-HRP (1:500 dilution in PBSTM) for 1 h at room temperature using a gentle rocking motion for the detection of target DNA. After washing with PBST/ Zn^{2+} , the chemiluminescent signal was captured by a CCD (charged-coupled device) camera after exposure to SuperSignal West Pico chemiluminescent substrate for 1 min. The intensity of each dot was quantified using the NIH ImageJ program.

Electrophoretic Mobility Shift Assay (EMSA)

Complementary pairs of forward and reverse oligonucleotides were annealed to obtain target dsDNAs. EMSA was performed with the Light Shift Chemiluminescent EMSA kit according to the manufacturer's protocol (Thermo fisher). Binding reactions were performed for 1 hr at room temperature and 30 min at 4°C in ZBA containing 150 mM KCl, 5 mM DTT, 10 % glycerol, 0.1 mg/ml BSA, 0.05 % NP-40, 0.25 nM target DNA and purified ZFPs. Gel electrophoresis was performed on a 10% native polyacrylamide gel in 0.5 $\times$ TBE buffer. After blotting on a nylon membrane, the DNA was cross-linked by a UV crosslinker for 4 min after 2 min pre-heating of the instrument. The chemiluminescent signal was captured by a CCD camera after exposure to Super Signal West Pico chemiluminescent substrate for 5 min.

Results and discussion

A ZF domain provides a versatile scaffold to be engineered to recognize any desired DNA sequences.^{28, 29} Two pairs of ZFPs (SEB400/SEB435 and Stx2-233/Stx2-268) were engineered to recognize *seb* gene and *stx2* gene encoding for staphylococcal enterotoxin B (SEB) and shiga toxin (Stx2), respectively released by *S. aureus* and *E. coli* O157. Each ZFP pair consists of capture and detection probes. Fig. 1 illustrates our system for direct detection of pathogenic DNA utilizing engineered ZFPs. The sensitivity in ZFP-based detection system could be improved depending on a signalling label. To attach a signalling label to our system, the detection probe ZFP was chemically conjugated with biotin which has a strong affinity toward streptavidin (SA). SA-HRP conjugate was applied to our detection system, generating chemiluminescent signal.

The capture probes of each ZFP pair, SEB400 and Stx2-268, were immobilized on the nitrocellulose membrane. Target dsDNA oligonucleotide was applied to the immobilized ZFP and bound by its respective capture ZFP probe. The biotin-conjugated ZFP detection probe was applied to the bound complex of the capture ZFP and target dsDNA to bind to the rest of the continuous target site of 36 bp. Fig. 2 shows the results of the chemiluminescent assay with the ZFP pair SEB400 and SEB435 targeting the sequence from 400 to 435 in *seb* gene (Fig. S-1). This ZFP pair was sensitive enough to detect 0.5 nM of DNA equivalent to 50 fmol as shown in Fig. 2 (a). Additionally, the DNA-dose dependent response was linear when a DNA dose was in log scale and quantitative in the range of 0.5 nM to 10 nM (R^2 correlation coefficient value of 0.96). Different levels of the capture ZFP SEB400 were immobilized on a nitrocellulose membrane (Fig. 2 (b)). Fig. 2 (b) shows that the target DNA was detected with an increasing concentration of the immobilized ZFP capturing more of the target DNA. Another ZFP pair Stx2-233 and Stx2-268 was designed to recognize the sequence from 233 and 268 in *stx2* (Fig. S-1). As shown in Fig. 3 (a), the limit of detection for this ZFP pair was determined to be 0.5 nM of DNA equivalent to 50 fmol. As in the ZFP pair SEB400/SEB435, we also observed a DNA-dose dependent response that was linear in log scale of a DNA dose and quantitative with R^2 of 0.99 (Fig. 3 (a)). As shown in Fig. 3 (b), target DNA was detected in proportional to the concentration of the capture probe ZFP Stx2-233 immobilized on the surface.

DNA binding specificity of the engineered ZFPs was examined. We immobilized the ZFP capture probes SEB400 and Stx2-268 on a nitrocellulose membrane. Each capture probe was incubated with its own target DNA and non-target DNA. As shown in Fig. 4 (a), signal intensity was observed only where the ZFPs were incubated with their own target DNA. Thus, each pair of the ZFPs was able to distinguish its own target DNA from the non-target DNA, showing high specificity. To demonstrate multiplexed detection, we applied a mixture of the two target DNA oligonucleotides onto our system with the capture ZFP of each pair immobilized. Incubation of a mixture of the two target DNAs showed high signal intensity at spots where either of the two capture ZFPs was immobilized (Fig. 4 (b)). Each pair of the ZFPs bound to its own target DNA in the presence of non-target DNA oligonucleotide. This result suggests that multiple engineered ZFPs can be immobilized on the surface for multiplexed detection of different pathogens simultaneously.

To investigate how differently the capture and detection ZFP probes might affect the sensitivity and specificity of our detection system, DNA binding affinity of the engineered ZFPs was measured by EMSA. EMSA is a quantitative method to determine the binding affinity of DNA-binding proteins. The K_d values of ZFPs ranged from 0.3 nM to 3.6 nM, which is consistent with K_d values of ZFPs previously reported.^{10, 18, 30–32} The K_d values of ZFP Stx2-268 and Stx2-233 (1.98 nM and 3.6 nM, respectively) are similar whereas those of ZFP SEB400 and SEB435 (2.3 nM and 0.3 nM, respectively) differ by almost 8-fold (Fig. S-2). The binding affinities of both the capture probes SEB400 and Stx2-268 are similar to each other, being 2.3 nM and 1.98 nM, respectively. However, the binding affinity of their respective detection probes SEB435 and Stx2-233 is 12-fold different. This difference might be attributed to a better capability of retaining target DNA tightly, resulting in slightly higher signal intensity of the ZFP SEB pair at varying DNA concentrations, compared to the ZFP Stx2 pair.

As demonstrated previously^{9–11} as well as in this study, the use of ZFPs may lead to significant contribution to application of DNA-binding domains for developing new technology for pathogen detection. ZFP construction by modular assembly described in this study is relatively easy, thus the use of ZFPs for a real-world diagnostic can be cost-effective. TALE (Transcriptional activator-like effector) DNA-binding domains can be also engineered to bind specific DNA sequences for potential diagnostic application. Compared to ZFPs, TALEs have more predictable DNA specificity and flexible programmability, which may confer favorable characteristics for diagnostic application. Thus, as a potential alternative, TALEs may further increase the potential of our system for pathogen detection.

Conclusions

This study has demonstrated the direct detection of pathogen-specific dsDNA in a multiplexed format utilizing custom-designed ZFPs. Each ZFP pair was engineered to recognize the virulent DNA sequence present in a specific pathogenic bacteria, providing high specificity and affinity for target DNA. Our system allows for multiplexed detection of pathogenic DNA with their respective ZFP pair in a single assay. In addition, our non-PCR based approach does not require additional steps involved in DNA denaturation and hybridization. We expect that this sensing system can be expanded for multiplexed detection of pathogens, suitable for POC diagnostic application. Our future study will be focused on combining this ZFP-based system with a fluorescent detection method to improve sensitivity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This research was supported by the National Science Foundation (NSF) – International Research Experience for Students (IRES) under grant number IIA-1358222 and an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number 5P20GM103436.

Notes and references

1. Hobson NS, Tothill I, Turner AP. Biosens. Bioelectron. 1996; 11:455–477. [PubMed: 8729237]
2. Lazcka O, Del Campo FJ, Munoz FX. Biosens. Bioelectron. 2007; 22:1205–1217. [PubMed: 16934970]
3. Pollock FJ, Morris PJ, Willis BL, Bourne DG. Appl. Environ. Microbiol. 2010; 76:5282–5286. [PubMed: 20525865]
4. Song J, Li PE, Gans J, Vuyisich M, Deshpande A, Wolinsky M, White PS. Adv. Exp. Med. Biol. 2010; 680:455–464. [PubMed: 20865530]
5. Yu S, Chen W, Wang D, He X, Zhu X, Shi X. FEMS Microbiol. Lett. 2010; 307:65–71. [PubMed: 20402781]
6. Amagliani G, Omiccioli E, Brandi G, Bruce IJ, Magnani M. Food Microbiol. 2010; 27:580–585. [PubMed: 20510774]
7. Abe K, Kumagai T, Takahashi C, Kezuka A, Murakami Y, Osawa Y, Motoki H, Matsuo T, Horiuchi M, Sode K, Igimi S, Ikebukuro K. Anal. Chem. 2012; 84:8028–8032. [PubMed: 22894566]
8. Wetmur JG. Crit. Rev. Biochem. Mol. Biol. 1991; 26:227–259. [PubMed: 1718662]

9. Ooi AT, Stains CI, Ghosh I, Segal DJ. *Biochem.* 2006; 45:3620–3625. [PubMed: 16533044]
10. Kim MS, Stybayeva G, Lee JY, Revzin A, Segal DJ. *Nucleic Acids Res.* 2011; 39:e29. [PubMed: 21134909]
11. Noh S, Ha DT, Yang H, Kim MS. *Analyst.* 2015; 140:3947–3952. [PubMed: 25969923]
12. Osawa Y, Ikebukuro K, Motoki H, Matsuo T, Horiuchi M, Sode K. *Nucleic Acids Res.* 2008; 36:e68. [PubMed: 18502777]
13. Stains CI, Porter JR, Ooi AT, Segal DJ, Ghosh I. *J. Am. Chem. Soc.* 2005; 127:10782–10783. [PubMed: 16076155]
14. Lee MS, Gippert GP, Soman KV, Case DA, Wright PE. *Science.* 1989; 245:635–637. [PubMed: 2503871]
15. Segal DJ, Beerli RR, Blancafort P, Dreier B, Effertz K, Huber A, Kokscho B, Lund CV, Magnenat L, Valente D, Barbas CF 3rd. *Biochem.* 2003; 42:2137–2148. [PubMed: 12590603]
16. Dreier B, Beerli RR, Segal DJ, Flippin JD, Barbas CF 3rd. *J. Biol. Chem.* 2001; 276:29466–29478. [PubMed: 11340073]
17. Liu Q, Segal DJ, Ghiara JB, Barbas CF 3rd. *Proc. Natl. Acad. Sci. U S A.* 1997; 94:5525–5530. [PubMed: 9159105]
18. Beerli RR, Segal DJ, Dreier B, Barbas CF 3rd. *Proc. Natl. Acad. Sci. U S A.* 1998; 95:14628–14633. [PubMed: 9843940]
19. Pavletich NP, Pabo CO. *Science.* 1991; 252:809–817. [PubMed: 2028256]
20. Porter JR, Stains CI, Jester BW, Ghosh I. *J. Am. Chem. Soc.* 2008; 130:6488–6497. [PubMed: 18444624]
21. Remy I, Michnick SW. *Biotechniques.* 2007; 42:137–139. 141 passim. [PubMed: 17373475]
22. Lewkowich IP, Campbell JD, HayGlass KT. *J. Immunol. Methods.* 2001; 247:111–118. [PubMed: 11150542]
23. Wang X, Zhang QY, Li ZJ, Ying XT, Lin JM. *Clin. Chim. Acta.* 2008; 393:90–94. [PubMed: 18402780]
24. Dungchai W, Siangproh W, Lin JM, Chailapakul O, Lin S, Ying X. *Anal. Bioanal. Chem.* 2007; 387:1965–1971. [PubMed: 17115138]
25. Dodeigne C, Thunus L, Lejeune R. *Talanta.* 2000; 51:415–439. [PubMed: 18967873]
26. Wang X, Chen H, Lin JM, Ying X. *Int. J. Biol. Sci.* 2007; 3:274–280. [PubMed: 17505537]
27. Bhakta MS, Segal DJ. *Methods Mol. Biol.* 2010; 649:3–30. [PubMed: 20680825]
28. Beerli RR, Barbas CF 3rd. *Nat. Biotechnol.* 2002; 20:135–141. [PubMed: 11821858]
29. Wolfe SA, Nekludova L, Pabo CO. *Annu. Rev. Biophys. Biomol. Struct.* 2000; 29:183–212. [PubMed: 10940247]
30. Sander JD, Zaback P, Joung JK, Voytas DF, Dobbs D. *Nucleic Acids Res.* 2009; 37:506–515. [PubMed: 19056825]
31. Liu Q, Xia Z, Zhong X, Case CC. *J. Biol. Chem.* 2002; 277:3850–3856. [PubMed: 11726671]
32. Segal DJ, Dreier B, Beerli RR, Barbas CF 3rd. *Proc. Natl. Acad. Sci. U S A.* 1999; 96:2758–2763. [PubMed: 10077584]

Fig. 1. Schematic representation of chemiluminescent detection of double-stranded DNA using engineered ZFPs chemically conjugated with biotin. The capture ZFP is immobilized on a nitrocellulose membrane and dsDNA is applied to its respective capture ZFP probe. After washing, biotinylated detection ZFP probe is applied to the bound complex of ZFP-DNA. Upon binding, ZFP-based detection is transduced into chemiluminescent signal via SA-HRP assay.

Fig. 2. Chemiluminescent detection of target dsDNA with an engineered ZFP pair SEB400 and SEB435. (a) Image of a chemiluminescent assay and a DNA dose-dependent plot. (b) Image of a chemiluminescent assay at varying concentrations of an immobilized ZFP SEB400 and a protein concentration-dependent plot. Data presented in this figure are representative of three independent experiments.

(a)

DNA [nM]

0.5

1

5

10

(b)

ZFP [nM]

100

150

200

400

800

Fig. 3. Chemiluminescent detection of target dsDNA with an engineered ZFP pair Stx2-233 and Stx2-268. (a) Image of a chemiluminescent assay and a DNA dose-dependent plot. (b)

Image of a chemiluminescent assay at varying concentrations of an immobilized ZFP Stx2-268 and a protein concentration-dependent plot. Data presented in this figure are representative of three independent experiments.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Fig. 4. Chemiluminescent detection of pathogen-specific DNA using their respective ZFP pairs. (a) DNA binding specificity of engineered ZFP pairs. (b) Multiplexed detection of target dsDNA using each ZFP pair. A mixture of two target DNAs for both ZFP pairs is incubated on a ZFP-coated nitrocellulose membrane. Data presented in this figure are representative of three independent experiments.