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A Multiplexed Transcription Activator-like Effector System for Detecting Specific DNA Sequences

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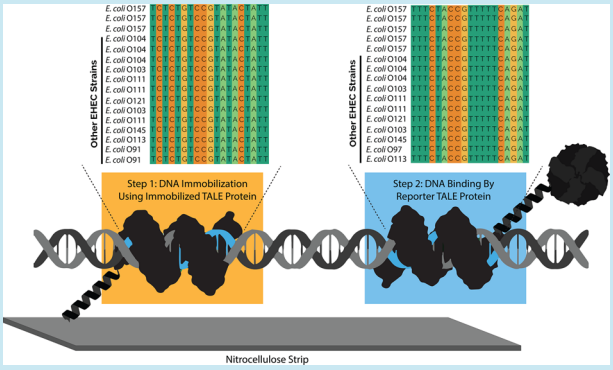
S *Supporting Information*

ABSTRACT: Transcription activator-like effectors (TALEs), originating from the *Xanthomonas* genus of bacteria, bind to specific DNA sequences based on amino acid sequence in the repeat-variable diresidue (RVD) positions of the protein. By altering these RVDs, it has been shown that a TALE protein can be engineered to bind virtually any DNA sequence of interest. The possibility of multiplexing TALEs for the purposes of identifying specific DNA sequences has yet to be explored. Here, we demonstrate a system in which a TALE protein bound to a nitrocellulose strip has been utilized to capture purified DNA, which is then detected using the binding of a second distinct TALE protein conjugated to a protein tag that is then detected by a dot blot. This system provides a signal only when both TALEs bind to their respective sequences, further demonstrating the specificity of the TALE binding.



The detection of specific DNA sequences is of great importance. It has been previously used to identify cancerous tissue, the presence of certain infections in patients, and to identify contaminants in our food supply.[1,2] Systems that bind to DNA and provide a readable output without the requirement for DNA amplification have been highly sought after, and progress has been seen most prominently through systems utilizing DNA hybridization reactions.[1]

Transcription activator-like effectors (TALEs) have recently come to the forefront of protein engineering partially because of their ability to bind to specific DNA sequences and the ease with which these proteins can be altered to bind unique sequences.[3,4] Each nucleotide of DNA with which a TALE interacts is determined by the specific amino acids in the proteins repeat-variable diresidue (RVD) region of the TALE.[3] By changing the amino acid sequence in the RVD it is possible to engineer the TALE to bind any DNA sequence of interest. Applications involving the fusion of TALEs to the nuclease subunit of FokI have created engineered proteins capable of cleaving any target sequence, a technology that is now employed in genome engineering.[5] However, there are very few applications that employ TALEs, or their fusions, for the rapid identification of DNA sequences in a sample.

By creating a TALE fusion with a protein tag recognizable by an antibody, we have built a system that can report on the presence of a specific DNA sequence without the need for PCR amplification of the DNA present in the sample. The inclusion of a second TALE without the protein tag provides increased specificity by increasing the length of specific DNA sequence resulting in fewer false positive results. By immobilizing this system on a nitrocellulose strip, the system becomes a more versatile test, which could be conducted virtually anywhere.

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MATERIALS AND METHODS

See Supporting Information for detailed methodology.

Culturing, Genetics, Protein Purification, and Blot Preparation. Strains were grown on Lysogeny broth with 30 μg/mL chloramphenicol and induced with 0.1 mM IPTG. Genes were designed *in silico*, synthesized with LacI inducible promoters, ligated into pBS1C3 and transformed into *Escherichia coli* BL21(DE3). Cells were grown to an OD_{600} of 0.6, lysed, and clarified by centrifugation at 18 000 rpm. His-tagged proteins were purified with an ÄKTA Protein Purification System (GE Life Sciences) using a HisTrap column. Purified TALE-B protein was blotted onto nitrocellulose, blocked with skim milk, and washed with TBS-T.

TALE Binding Specificity on Nitrocellulose. TALE-B nitrocellulose blots were submerged for 90 min in binding buffer containing 1.66 mM 6-carboxyfluorescein-labeled (FAM-labeled) TALE-B target sequence and 1.96 mg/mL salmon sperm DNA. Blots were imaged on a FujiFilm LAS-4000 chemiluminescent camera.

Dual TALE Capture. TALE-B nitrocellulose blots were submerged in 1.66 mM TALE target plasmid (BBa_K1189006) and binding buffer for 90 min, and then soaked in 120 μg of purified TALE-A+β-lactamase (BBa_K1189031) in binding buffer for 1 h. Blots were soaked in β-lactamase primary antibody (AB12251, Abcam) at 1:5000 overnight at 4 °C in skim milk and then soaked with antimouse HRP conjugated antibody at 1:20 000 (172−1011, Bio-Rad) in skim milk. Blots were rinsed with TBS-T, developed with ECL substrate (Promega), and imaged on a chemiluminescent camera.

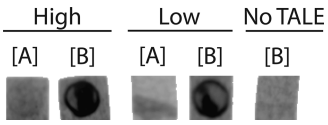

Figure 1. TALE-B is specific to the [B] target sequence. TALE B was immobilized on strips of nitrocellulose paper. The strips were then exposed to high (1.66 mM) and low (1 mM) concentrations of both the target sequence [B] and nontarget sequence [A]. A negative control (No TALE) where no protein was immobilized on the nitrocellulose showed no background due to FAM-labeled DNA when exposed to the 1.66 mM concentration of [B] target sequence. In addition to the FAM-labeled DNA, all the DNA solutions used in this experiment contained 1.96 mg/mL of salmon sperm DNA to create a mixed population of DNA. In the presence of this mixed population TALE-B was capable of binding to the FAM-labeled target DNA when it was present.

RESULTS

TALE Proteins Can Be Immobilized and Retain Target Specificity. We observed the ability of immobilized engineered TALE proteins on nitrocellulose paper to bind target DNA that was labeled with 6-carboxyfluorescein (FAM), a fluorescent probe. Successfully captured FAM-labeled DNA was visualized as blots by chemiluminescence (Figure 1). We observed that the specificity of the engineered TALE was intact when immobilized, as the TALE-B only bound to the FAM-labeled TALE-B target sequence, denoted as [B], while no binding was observed when tested with the TALE-A FAM labeled target sequence, denoted as [A]. We also observed that immobilized TALEs were capable of functioning in mixed populations of DNA as blots were supplemented with salmon sperm DNA while retaining their ability to capture the appropriate target sequence.

Capturing Target DNA with Two TALEs. We were able to capture a DNA plasmid containing both TALE target sequences (referred to as [A] and [B]). A purified plasmid containing both TALE [A] and [B] target sequences to a nitrocellulose strip with immobilized TALE-B. Subsequent exposure of TALE-A with the β-lactamase protein tag formed an area of β-lactamase observable only in the presence of TALE-B, the plasmid with both [A] and [B] target sequences, and TALE-A with the fused β-lactamase. This indicates all components of the system must be present to detect a signal. In the samples where all components of the system were present we observed a signal when probed with the primary and secondary antibody in the dot blot (Figure 2).

DISCUSSION

We have developed a novel system to detect specific sequences of DNA. By using a two TALE system to increase specificity, it may be possible to reduce false-positive outputs while detecting the conserved pathogenic sequences of interest, and testing time could be reduced as current PCR amplification methods would not be required.

While the results shown here are a proof of concept, the advent of rapid DNA synthesis technologies will reduce the

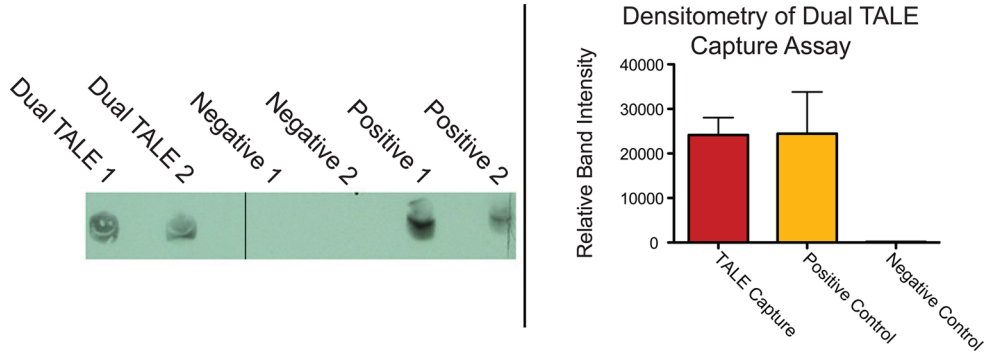

Figure 2. Dual TALE capture system is able to trap DNA and generate a signal. (Left) TALE-B was immobilized on nitrocellulose strips and subsequently soaked in a solution containing a plasmid DNA with both [A] and [B] sequences. Next, the strips were soaked in a solution of TALE-A:β-lactamase fusion protein. Finally, the strips were visualized by a dot blot assay using a primary antibody specific to the β-lactamase epitope fused to TALE-A. Strip Negative 1 was not exposed to the plasmid DNA solution, and strip Negative 2 was not exposed to the TALE-A:β-lactamase fusion protein. The Positive strip was immobilized TALE-A:β-lactamase fusion protein. (Right) Quantitative densitometry analysis of the dot blot assay, showing the two test strips giving a similar response to the positive controls.

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time and cost needed to further develop these types of systems. Although further research and development is required there is potential to make this method of DNA detection mobile and rapid. This could open new avenues for testing at point-of-care applications or those that lack sophisticated diagnostics machinery.

■ ASSOCIATED CONTENT

Supporting Information

A detailed description of the methods used and of the FAM-labeled sequences for TALE targets [A] and [B]. This material is available free of charge *via* the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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