Paired design of dCas9 as a systematic platform for the detection of featured nucleic acid sequences in pathogenic strains _si_001

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Supporting Information

Paired design of dCas9 as a systematic platform for the detection of featured nucleic acid sequences in pathogenic strains

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Supplementary Figure S1. Reconstitution of luciferase activity via rapamycin-induced FRB/FKBP dimerization. Error bars show standard error for three independent experiments.

Supplementary Figure S2. Protein purification and characterization. (a) SDS-PAGE of purified proteins. Cell lysate showed the efficiency of 200 M IPTG induction. (b) Characterization of PC reporter system using different protein concentrations. Error bars show standard error from three independent experiments.

a

b

Supplementary Figure S3. Array-based detection of B. subtilis genome using PC reporter system. (a) Markers selected from the genome. (b) Output of PC reporter using B. subtilis genomic DNA and E. coli genomic DNA.

a

b

Supplementary Figure S4. Distribution of scored segments from Mycobacterium tuberculosis genome.

Supplementary Figure S5. Amino acid sequences of all proteins.

FRB

ATCCTCTGGCATGAGATGTGGCATGAAGGCCTGGAAGAGGCATCTCGTTTGTACTTTGGGGAAAG

GAACGTGAAAGGCATGTTTTGAGGTGCTGGAGCCCTTGCATGCTATGATGGAACGGGGCCCCCAG

ACTCTGAAGGAAACATCCTTTAATCAGGCCTATGGTCGAGATTTAATGGAGGCCCAAGAGTGGTG

CAGGAAGTACATGAAATCAGGGAATGTCAAGGACCTCaccCAAGCCTGGGACCTCTATTATCATGT

GTTCCGACGAATCTCA

FKBP

GVQVETISPGDGRTFPKRGQTCVVHYTGMLEDGKKFDSSRDRNKPFKFMLGKQEVIRGWEEGVAQM

SVGQRAKLTISPDYAYGATGHPGIIPPHATLVFDVELLKLE

NFluc

MEDAKNIKKGPAPFYPLEDGTAGEQLHKAMKRYALVPGTIAFTDAHIEVDITYAEYFEMSVRLAEAMKR

YGLNTNHRIVVCSENSLQFFMPVLGALFIGVAVAPANDIYNERELLNSMGISQPTVVFVSKKGLQKILNV

QKKLPIIQKIIIMDSKTDYQGFQSMYTFVTSHLPPGFNEYDFVPESFDRDKTIALIMNSSGSTGLPKGVAL

PHRTACVRFSHARDPIFGNQIIPDTAILSVVPFHHGFGMFTTLGYLICGFRVVLMYRFEEELFLRSLQDY

KIQSALLVPTLFSFFAKSTLIDKYDLSNLHEIASGGAPLSKEVGEAVAKRFHLPGIRQGYGLTETTSAILIT

PEGDDKPGAVGKVVPFFEAKVVDLDTGKTLGVNQRGELCVRGPMIMSGYVNNPEATNALIDKDG

CFluc

SGYVNNPEATNALIDKDGWLHSGDIAYWDEDEHFFIVDRLKSLIKYKGYQVAPAELESILLQHPNIFDAGVAGLPDDDAGELPAAVVVLEHGKTMTEKEIVDYVAS

QVTTAKKLRGGVVFVDEVPKGLTGKLDARKIREILIKAKKGGKIAV

dCas9

DKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVE

EDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSA

RLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSAS

MIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFD

QSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAIL

RRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFI

ERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTV

KQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIE

ERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSL

TFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQ

KGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVD

AIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGG

LSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREI

NNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFK

TEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSD

KLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGY

KEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQ

LFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFD

TTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD

Supplementary Figure S6. DNA sequence of sgRNA expression cassette [Primer site, T7 promoter, BsaI restriction site, LacZ' (reversed), LacZ promoter (reserved), crRNA, T7 terminator].

CCACCTGACGTCTAAGAAACCATTATTATCATGACATTAACCTATAAAAATAGGCGTATCACGAGG

CAGAATTTCAGATAAAAAAAATCCTTAGCTTTCGCTAAGGATGATTTCTGGAATTCCGATCGTGACA

CAAGGTTTAATACGACTCACTATAGGCGAGACCCCATTCGCCATTCAGGCTGCGCAACTGTTGGG

AAGGGCGATCGGTGCGGGCCTCTTCGCTATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAG

GCGATTAAGTTGGGTAACGCCAGGGTTTTCCCAATCACGACGTTGTAAAACGACGGCCAGTGAGC

GCGCGTAATACGACTCACTATAGGGCGAATTGGGTACCGGGCCCCCCCTCGAGGTCCTCCAGCT

TTTGTTCCCTTTAGTGAGGGTTAATTGCGCGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGT

GAAATTGTTATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAAGCCTGG

GGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCGGTCTCAGTTTTAGAGCTAGAAAT

AGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTAG

CATAACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTGCGGCCGCTGCAGTCCGGCAAA

AAAGGGCAAGGTGTCACCACCCTGCCCTTTTTCTTTAAAACCGAAAAGATTACTTCGCGTTATGCA

GGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAGCTCAC TCAAAGGCGGTAATA

Supplementary Figure S7. DNA target sequence for Figure 1 and Figure 2 [sgRNA binding site 1, sgRNA binding site 2, Spacer (27 bp)].

ATCGATCCATCTTCAGGGAAAAGCTGGGTGTCGCCTGCAGAGAGATATGCAGTTGTTCCTGATGA

AACGGGCCTAACGGATGGTT

Supplementary Table S1. Parameters for BLAST.

Parameter	Description
evalue=10	ExpecPtation value (E) threshold for saving hits, Default
word_size=11	Word size for wordfinder algorithm (length of best perfect match)
reward=1	Reward for a nucleotide match
penalty=-3	Penalty for a nucleotide mismatch
gapopen=5	Cost to open a gap
gapextend=2	Cost to extend a gap
outfmt=5	BLAST output in format .XML

Supplementary Table S2. Markers used in array-based detection of M. tuberculosis H37Rv genome.

Region 1		Binding site 1	Binding site 2
Region 1	Marker 1	cccaaagctttgtcgacgccag	cctggtcacggacgttgctccgg
Region 1	Marker 2	ccgttgcataggtcaatgcaagt	caactaactaaccaaccaggttgg
Region 1	Marker 3	cctggccgtgtcggtcctctcac	atgaagaatatgcggagtctagg
Region 1	Marker 4	cctcttggtacggacatgcgcgc	ggatgcgaaaactacgtcctcgg
Region 1	Marker 5	ccgccaaaagcagaatggcagga	gacccgccgaaccggtttccgg
Region 1	Marker 6	cctgtttggccgtcctggctggc	atcggcaaaggaagcgtcgctgg
Region 1	Marker 7	cctactgtggacggcagccgtgg	tatgccgggcattgcctcaccgg
Region 1	Marker 8	ccgtacccggggaccagcccgac	tttcgtctgcgtcggactcttgg
Region 2		Binding site 1	Binding site 2
Region 2	Marker 1	ccttgtctcatgtttgccagcag	gactgccggggtcaactcggagg
Region 2	Marker 2	ccggtacaaagggctgcgatgcc	aagccggtctcagttcggatccgg
Region 2	Marker 3	ccgaggatttccgaatgggggaaa	ctacccgcatctgaatatatagg
Region 2	Marker 4	cccggctgagaggcagtcagaaa	ctgggatggtctgccgtagacgg
Region 2	Marker 5	cctaaatactcctcgatgaccga	gggaatggtgaaaaagtaccccg
Region 2	Marker 6	ccctactaggttactgacgtcag	tgtaaagcgtggcagtgagacgg
Region 2	Marker 7	ccgtacgtcgaaagggaaacagc	aagcgtgtgctaagtgggaaagg
Region 2	Marker 8	ccacccttgaaagagtgcgtaat	tgtgcgccgataatgtagcgggg
Region 3		Binding site 1	Binding site 2
Region 3	Marker 1	ccccagctgagtagtcggaggcc	gttcgcgcggacccatgtcgagg
Region 3	Marker 2	ccaccacttaatcagcaccaggc	tagtcaacctccaagtcagccgg
Region 3	Marker 3	ccacttgactagccactgccaac	acccggacccggactggatagg
Region 3	Marker 4	ccagccccattgtctctcatctg	tcgtcgtgcgtacggacacgg
Region 3	Marker 5	ccagcaagcgatcctgagcgtcg	ccctcggcgagccaggcactggg
Region 3	Marker 6	ccgacgctagttgccaggtccca	gcatcgggatcctttcggctcgg
Region 3	Marker 7	ccaccgcttgtgggatcaatcgt	gaatgccacgctcacttcgtcgg
Region 3	Marker 8	cctctgtgtgcaagtcgatgacg	ggatgtgcagtttccgggtcagg
Region 4		Binding site 1	Binding site 2
Region 4	Marker 1	ccaccgtggcgccttcgtcgcgc	gaccgacgcggagtgcaccgcgg
Region 4	Marker 2	ccgagtttggaaatgtgcaccag	cgatacaaaggcaccgaaatcgg
Region 4	Marker 3	ccttgggtccgatgacctccccg	ggtggtcacccgcggggcgtagg
Region 4	Marker 4	cctgcgaagggatcccgtcgagc	ggtgacgaagtctcttggtcccgg
Region 4	Marker 5	ccgaccggtctcgccggtggaga	atgtaccgcttcgatgtctccgg
Region 4	Marker 6	ccagctttttggtcagggacacgc	ttcgcgaccctcgtaggtgtcggg
Region 4	Marker 7	ccggatccaggctgagaatcgtc	cgcagcccgtcgacaaacgacgg

|Region 4|Marker 8|ccggccgcatacatgcgtcctc|agaagtcaagtttctttttgggg|

Supplementary Table S3. Markers used in array-based detection of B. subtilis genome.

Region 1		Binding site 1	Binding site 2
Region 1	Marker 1	ccttgaggaattgcagcatattg	aaacgaaacagaggacgccgcgg
Region 1	Marker 2	cctgttgtgcttcctggcgagga	gacgggaaagagcataatcaggg
Region 1	Marker 3	ccggacagggaaagccggatgaag	cattgagctgaagggagacccgg
Region 1	Marker 4	ccgtttcaaacttcaattgagc	gacagcacagtgtgtataagggg
Region 1	Marker 5	ccttgtacatgacggtgcccgtc	aattgacgaactgatcgcagagg
Region 1	Marker 6	cctacttataacttcgcggtagc	aaaatgacacacgtgctgcgcgg
Region 1	Marker 7	ccgggtttttagaagtgcgccctt	ttgaaatcagtattcaaaggcgg
Region 1	Marker 8	ccgccgttacaggctgaagttgg	tcaaacagagtggaaccgcgcgg
Region 2		Binding site 1	Binding site 2
Region 2	Marker 1	cctggcgttaagatagaagatga	accaagctgtgatttagttgcgg
Region 2	Marker 2	ccggttttagttaagaaactattt	aacatgcaaatcgccctccatgg
Region 2	Marker 3	ccttctgttgtggatcattccaa	acggaagggttaagaggtgtccgg
Region 2	Marker 4	ccacctataaaatgcggatcgtc	tgcagttttggtctccttacgagg
Region 2	Marker 5	ccaaaagggtcgatatacagattt	acttggccaagtgcagcatgggg
Region 2	Marker 6	ccgctacatgctttgtagttcatt	agatttgctgtaccatgaacgg
Region 2	Marker 7	cctttgtctaggaggaagctggcc	ttatatccattgtatttcaggcgg
Region 3		Binding site 1	Binding site 2
Region 3	Marker 1	ccgatcttcgcagttgcgaactg	tagaagcccatatggcgacgtgg
Region 3	Marker 2	ccatccgcatgagacctttagcg	caaattgagcgtacgtatacggg
Region 3	Marker 3	ccaagtaagttggccgcaggcac	tttccgctgtattcgaacccgcgg
Region 3	Marker 4	ccccatatacactagcttgatacac	tttgatagagaaagaacacaagg
Region 3	Marker 5	ccggtcgggtcatctgcggcgtt	ctgatgccgaatttgacatacgg
Region 3	Marker 6	ccgaccaagcccgtaagctgac	cggtttaatctgaacgataacgg
Region 3	Marker 7	cctgtatgccccgctggtttcggg	aatgaatgcttgctttgatgtgg
Region 3	Marker 8	ccttttctctgactgcttcaggc	ttaccggtatcgataggcaaagg
Region 4		Binding site 1	Binding site 2
Region 4	Marker 1	ccaatagagatggtaatgactag	tcactaaactgaatatactgagg
Region 4	Marker 2	ccgtcatacgtatacttaccaga	agaacgttacagtatctttctgg
Region 4	Marker 3	ccaaaatgatatgaggggctttgtc	atctggaaggatcactgaacggg
Region 4	Marker 4	ccggctgcacgggatctttggttt	cttgcgagggctttcttggcgg
Region 4	Marker 5	ccgatcatcggaccacgaacggt	aaaagccgttgaaatcagtctgg
Region 4	Marker 6	cctccattgatgaggggatgat	ggcatccaccatctttgtccgg
Region 4	Marker 7	ccataaccatgggattcatacca	tgctgagactgttatcatatagg
Region 4	Marker 8	ccaatacccttaccaaaacctcc	gtgatggtcaaggcaacaatagg
Region 5		Binding site 1	Binding site 2
Region 5	Marker 1	cctccttaacgttaattctcatc	caaaacgaagtagttcaaacagg
Region 5	Marker 2	ccatctcagtatctgaagaagga	ggatatttatttcatggctcagg
Region 5	Marker 3	cccttgcttactgcatcgtcga	gtcaccaatgcggaacgggcagg
Region 5	Marker 4	ccatctagtgtaacagaacatcc	gcgccgatatccaaaaatgccgg
Region 5	Marker 5	ccgcccgaacaagacttcattca	gtttctttgcgcgcggccaacagg
Region 5	Marker 6	ccgagtaacgcaaacatattgac	ggtcaaacggattgacgccaagg
	Marker 7	ccggctaaatagttcaagccgtc	tcaattgtcagttcatgtttagg
	Marker 8	ccgcatattggtatgctgggtta	caaaatctttgctgcatccagg
Region 6		Binding site 1	Binding site 2
Region 6	Marker 1	cccgtataatatgcgtcgcgctg	cgtcaatctgactcgaatccagg
Region 6	Marker 2	cctttctgagcacctgattaacc	tacctcttgtaaatactcgtgg

Region 6	Marker 3	cctcaacctttgcgtccgggtcc	atgttttgatattagttggctttgg
Region 6	Marker 4	ccagaaagctagttttatttgaa	tccttcaaaatggaccggaacgg
Region 6	Marker 5	ccttttctattgagaaaagagaa	tcagctattcttgtaaatatgagg
Region 6	Marker 6	ccaagcaatgaagccgtcagccgg	cgccgccgaggccggaacccgg
Region 6	Marker 7	ccacgagatatctaactgccata	gctgatcatgaatacaacgcgg
Region 6	Marker 8	cctttaatatggatgctgatgtc	gtggttatctgtatgtaagaagg
Region 7		Binding site 1	Binding site 2
Region 7	Marker 1	ccgaaagcgtaagaagtcctttgg	tgtatagcaagacttttggccgg
Region 7	Marker 2	ccgctggacgcacaatcatttgta	ggcatgggttttccgcctttggccgg
Region 7	Marker 3	ccgaacagttataacctgccaag	atgagtttcatcataatgtgcgg
Region 7	Marker 4	ccctgtattgtaaggaatatagc	aagcggctgaatagccgcgtagg
Region 7	Marker 5	ccggttccaaagtagccggatttc	tgataggaaaatccgtagccgg
Region 7	Marker 6	ccaagcttagggccgattgagac	gcatcaatggtgagctgaagagg
Region 7	Marker 7	ccaagacgttcaggtaactgaaa	gaaacgccgcgcgtcatttggg
Region 8		Binding site 1	Binding site 2
Region 8	Marker 1	cctcatccagtgggtaaacgga	agaaccgggcatctcctatacgg
Region 8	Marker 2	ccgaatacctgtgcatcaccgag	cgcgttccatgtcacttgagccgg
Region 8	Marker 3	ccttcttggaacttcacacctga	gcttatgctcaagttgcgctagg
Region 8	Marker 4	ccaaggctcacaaatgcagacaa	ccaatgcagcagggacagcaagg
Region 8	Marker 5	ccgccgagcggggtgatcgctcc	acttagaatataagcttccgg
Region 8	Marker 6	cctgaagatacttatcaggtatt	cctgaagatacttatcaggtatt
Region 8	Marker 7	cctcacccttttgattcttctt	aaaggccagaccgccatttgcgg
Region 8	Marker 8	ccaccacaagccggctgggcttt	gcctttcaaacactgaatgacgg
Region 9		Binding site 1	Binding site 2
Region 9	Marker 1	cctattgttaaaggagatatacaa	tcatttttatccgaatataagg
Region 9	Marker 2	cctcgctcacgacggtaagagtt	tttttgcttcctcaaggcgaagg
Region 9	Marker 3	ccagcttcttaatcgtcagccgc	atgcaattgaggcatagctatgg
Region 9	Marker 4	ccgtgtgtatgtccgtttttcgc	cattagcaaaccccttcctttgg
Region 9	Marker 5	ccgtcagcagttggtgaggctt	cctccttttataggcgtctgcgg
Region 9	Marker 6	ccgcttatcccaagtccaaaattt	ctcttcaataggaccgattctgg
Region 9	Marker 7	cctgaaacgttccgactgaaaac	ttgttcataattcgaatataagg
Region 9	Marker 8	ccccaagccgtcatgtcaataaaa	aatgcttgagtagattgctcagg