
Supplementary materials

Detection of tobacco bacterial wilt caused by *Ralstonia solanacearum* by combining polymerase chain reaction with α -hemolysin nanopore

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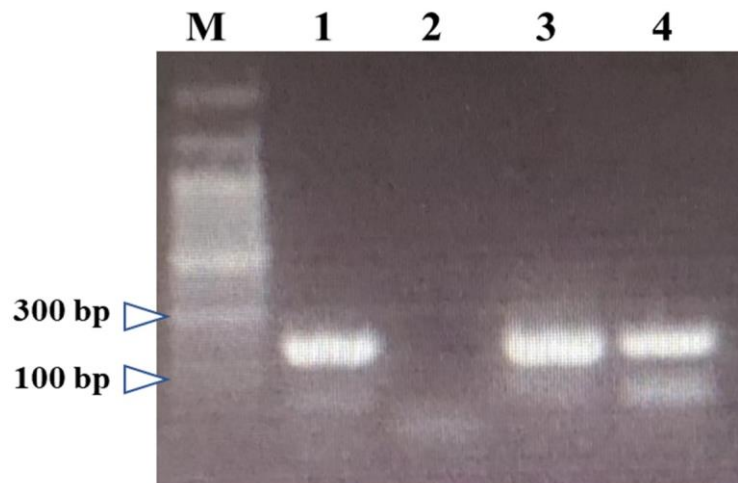


Figure S1. 2% Agarose gel electrophoresis of the PCR product. Lane 1, DNA Ladder L(100~3000bp). Lane 2, PCR product of pure water (negative control). PCR product of solution in *cis* chamber (lane 3) and *trans* chamber (lane 4). Strand A in the two chambers were purified by the Universal DNA Purification kit, which was used as the template for PCR.

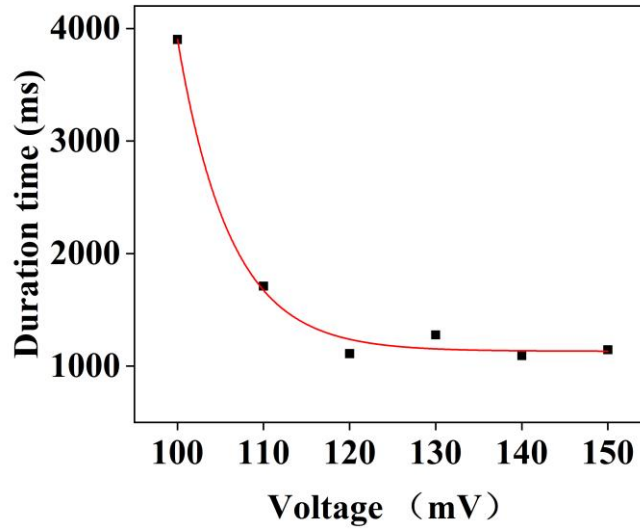


Figure S2. Durations of the synthetic strand A were decreased as the voltage increased from 100 to 150 mV.

To determine whether strand A was translocated, a test of the relationship between duration and voltage was performed. Strand A was synthesized and purified by Takara Bio Inc. The results show duration of strand A was decreased from 3902.64 ms to 1145.51 ms as the voltage increased from + 100 to + 150 mV. At the end of the translocation test, the solution in the two chambers were purified by the Universal DNA Purification kit, which were used as the templates for PCR. The results show that the 198 nt fragments were observed on a 2% agarose gel. These experimental results verified that strand A was translocated to the *trans* chamber.

Duration of the unpurified lambda exonuclease-treated PCR products containing strand A ranged from 20.11 ms to 7232.85 ms at + 100 mV voltage (Figure 3). However, duration of the synthesized strand A was between 52.03ms-35364.35 ms at + 100 mV voltage. We speculated that different buffer solution of purified and unpurified strand A may lead to different higher structures, and thus different durations. In addition, strand A may interact with other ions in the lambda exonuclease- PCR buffer or TE buffer, which cause different non-covalent interactions between strand A and nanopores, including electrostatic interactions, π -effect, and hydrophobic interactions.

In addition, the lambda exonuclease-treated PCR products were purified by the Universal DNA Purification Kit to obtain relatively pure strand A. The voltage results show duration of strand A was decreased from 4545.95 ms to 518.84 ms as the voltage increased from + 100 mV to + 130 mV, and duration of the purified strand A was between 39.8 ms-101000 ms at + 100 mV. Duration of the purified lambda exonuclease-treated PCR product was similar to that of the synthetic strand A, which was significantly longer than that of the unpurified lambda exonuclease-treated PCR products.

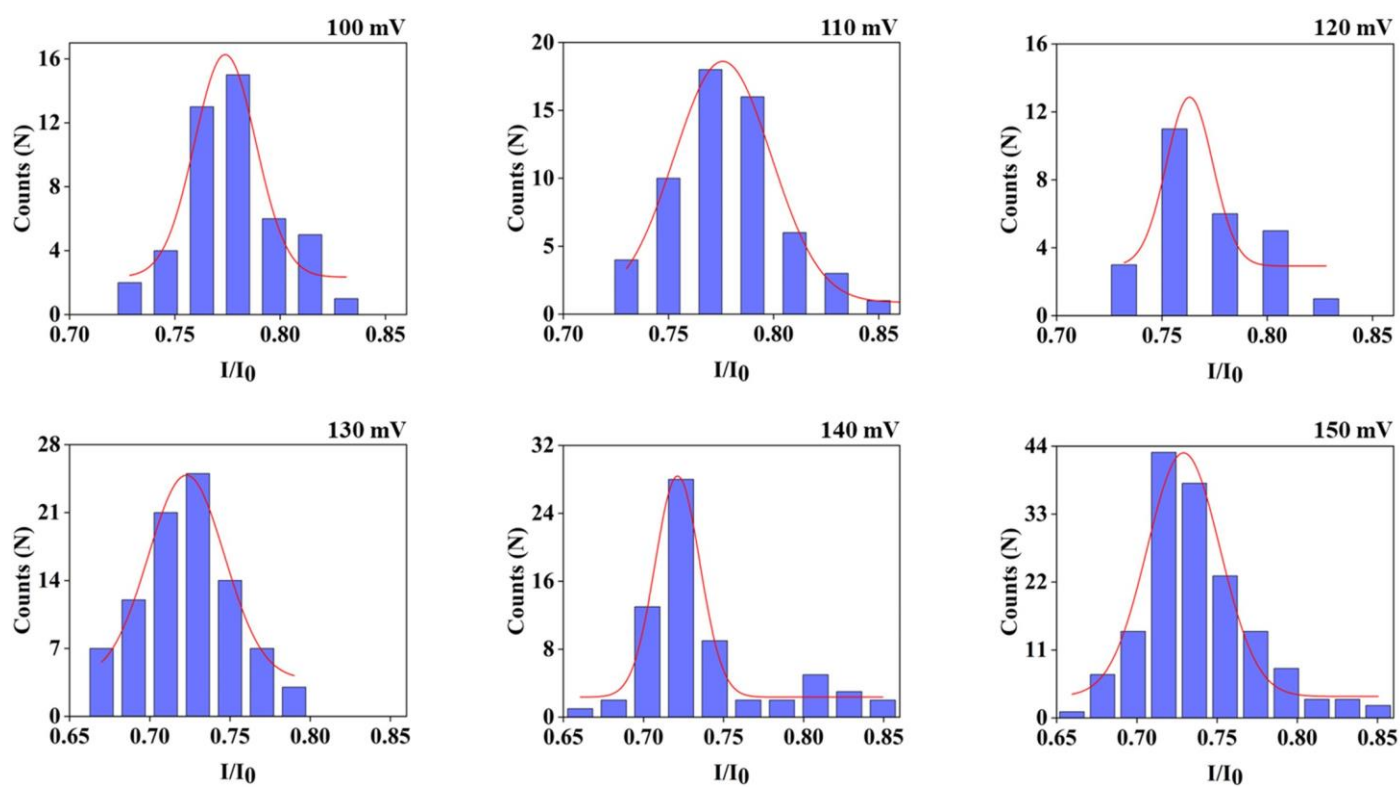


Figure S3. I/I_0 histogram of events of the strand A from +100 mV to +150 mV in 0.75 M/3 M (*cis/trans*) KCl.

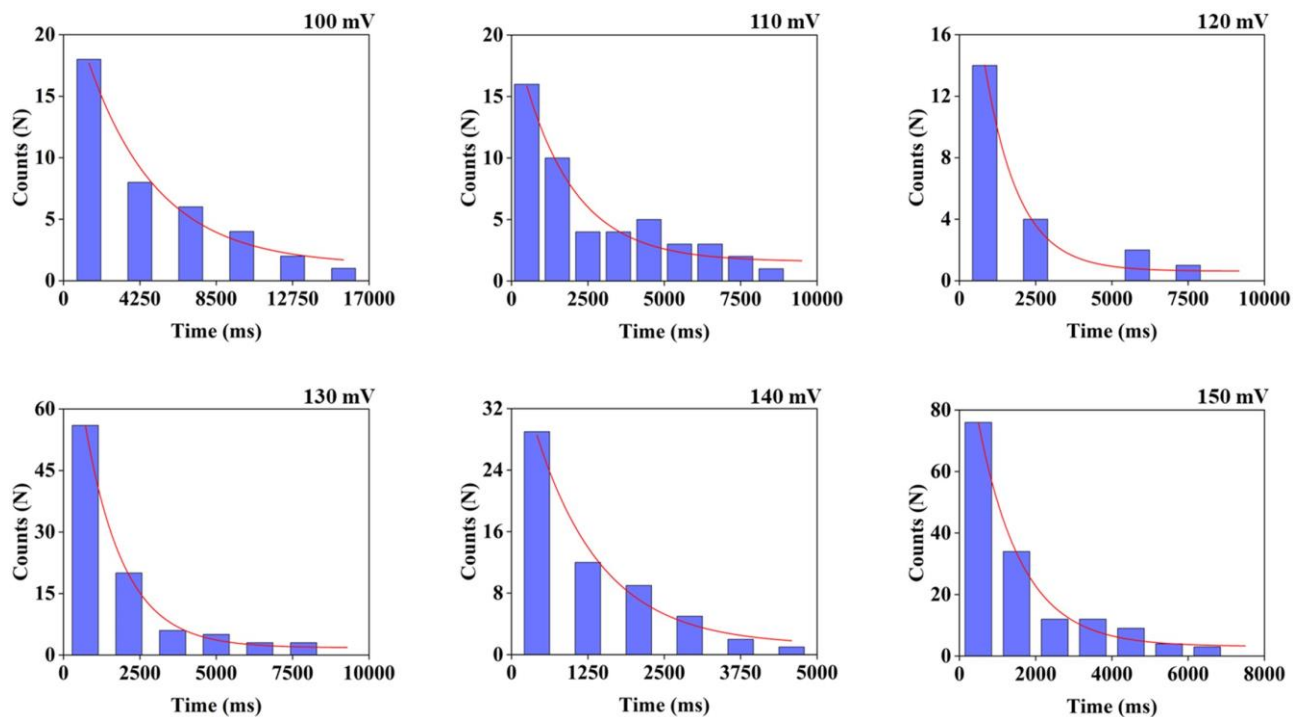


Figure S4. Duration histogram of events generated by strand A from +100 mV to +150 mV in 0.75 M/3 M (*cis/trans*) KCl.

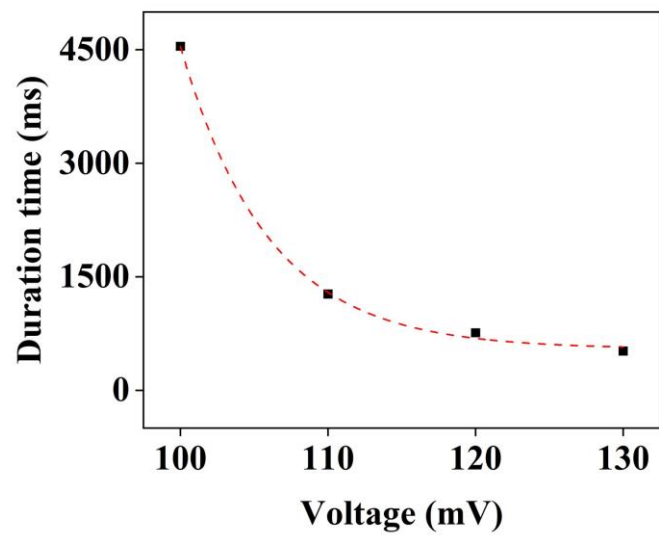


Figure S5. Durations of the Universal DNA Purification kit-purified strand A were decreased as the voltage increased from 100 to 130 mV.

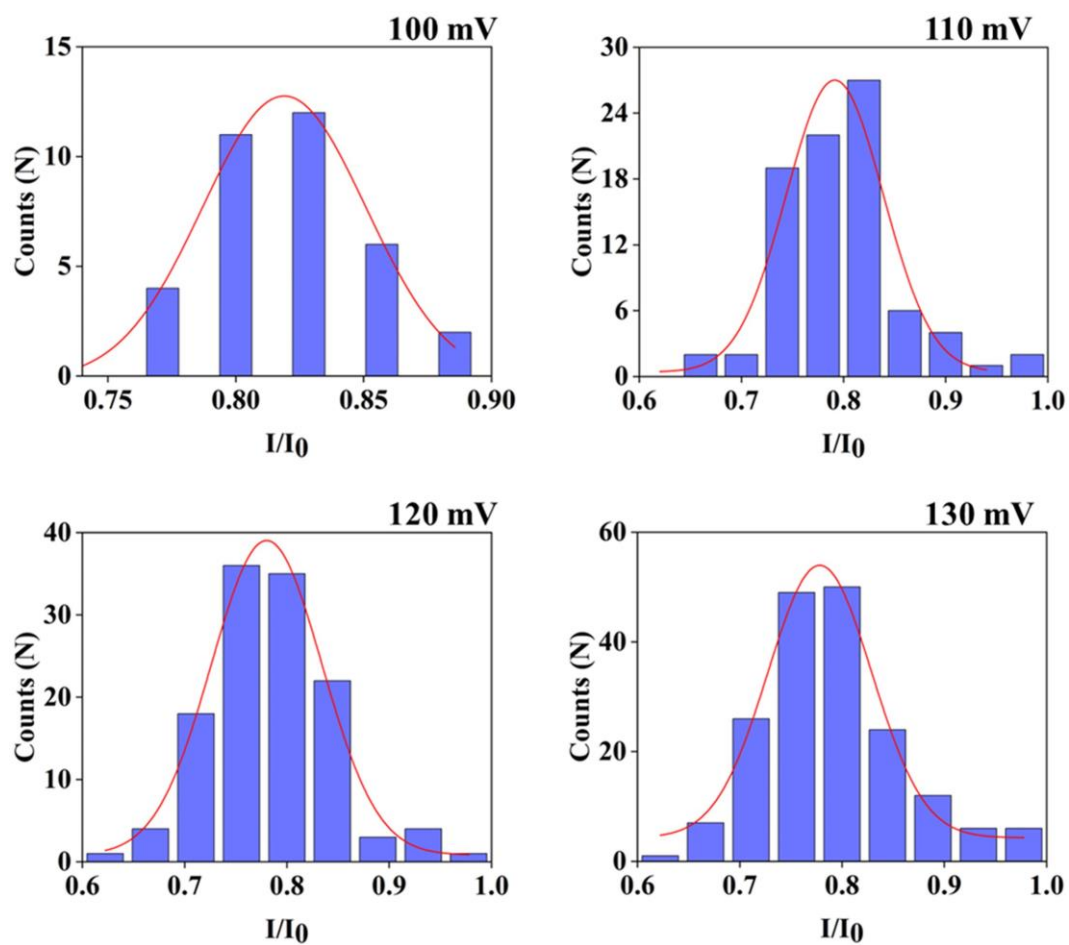


Figure S6. I/I_0 histogram of events generated by the Universal DNA Purification kit-purified strand A under gradient voltage from +100 mV to +130 mV in 0.75 M/3 M (*cis/trans*) KCl.

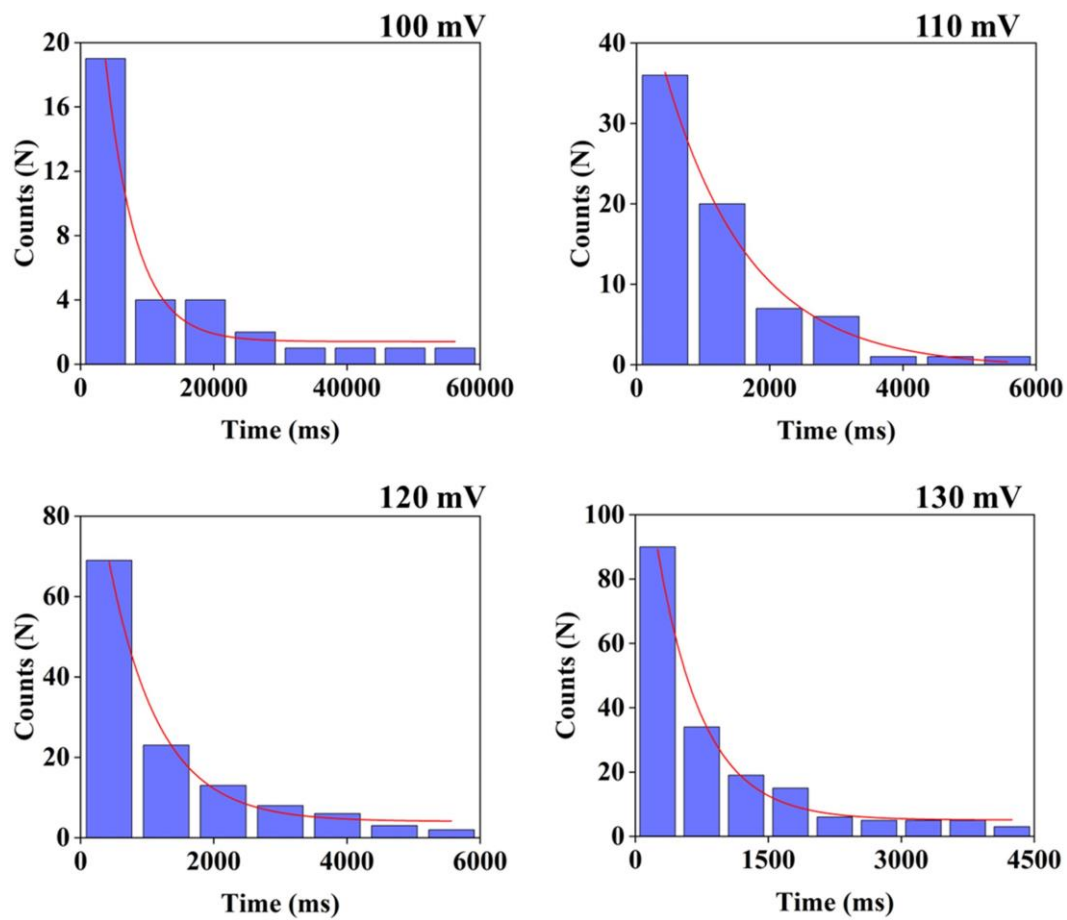


Figure S7. Duration histogram of events generated by the Universal DNA Purification kit-purified strand A under voltage from +100 mV to +130 mV in 0.75 M/3 M (*cis/trans*) KCl.

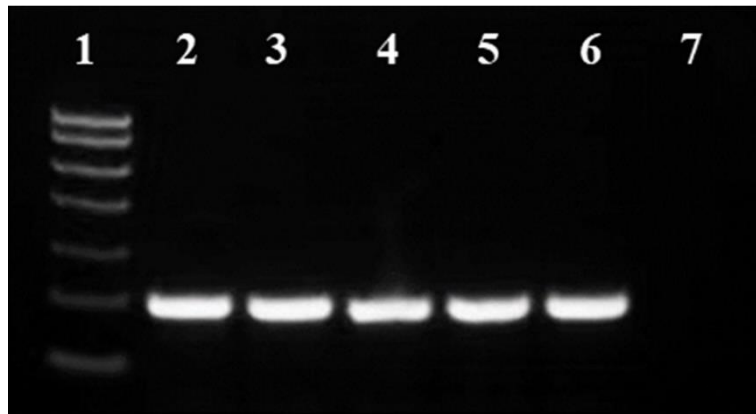


Figure S8. 2% Agarose gel electrophoresis of the PCR product. Lane 1, *Trans* DNA Marker I. Lane 2, PCR product of *R. solanacearum* genome. Lane 3-7, PCR product of field tobacco samples of 1-5.

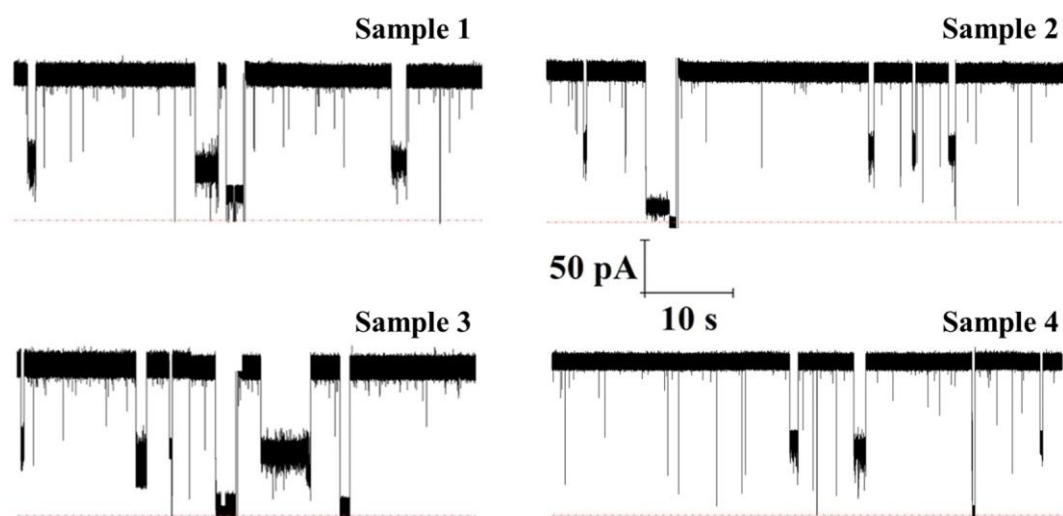


Figure S9. Current trace of the 4 positive field samples. All nanopore data were recorded at +100 mV in 0.75 M/3 M (*cis/trans*) KCl, and the same below.

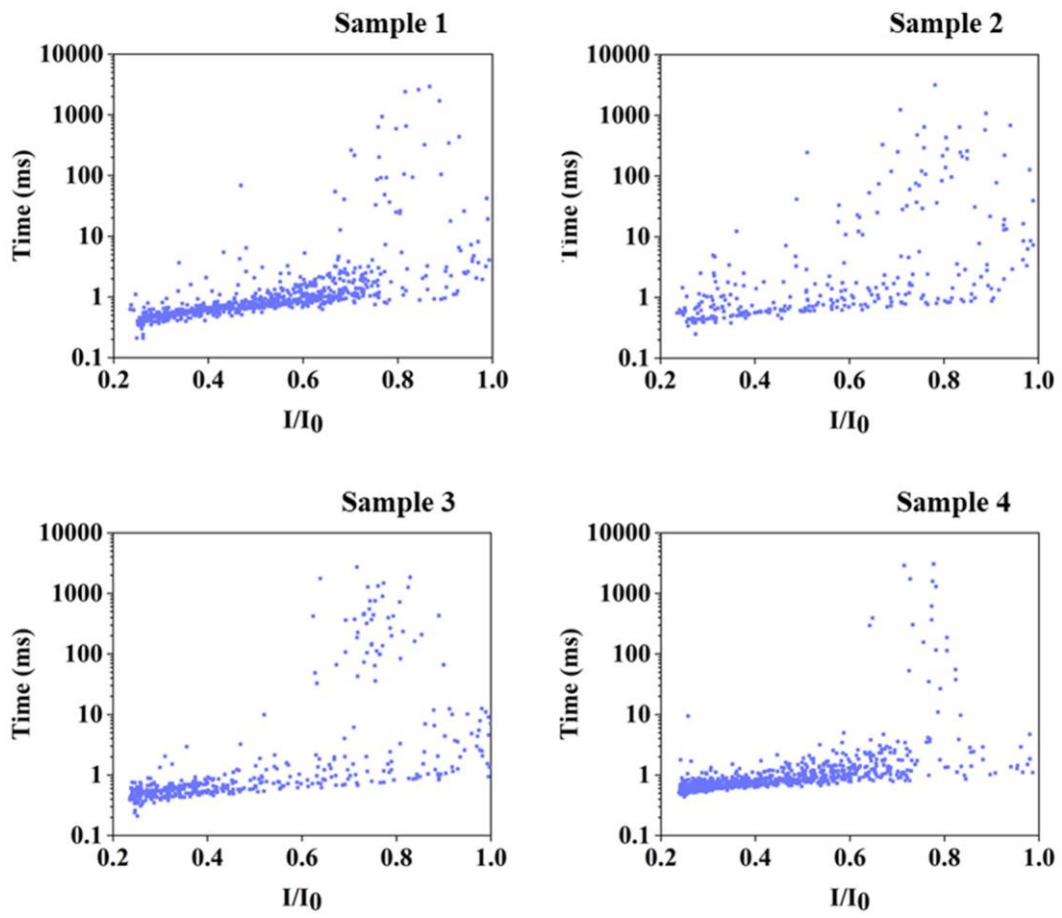


Figure S10. Scatter plot of the 4 positive field samples.

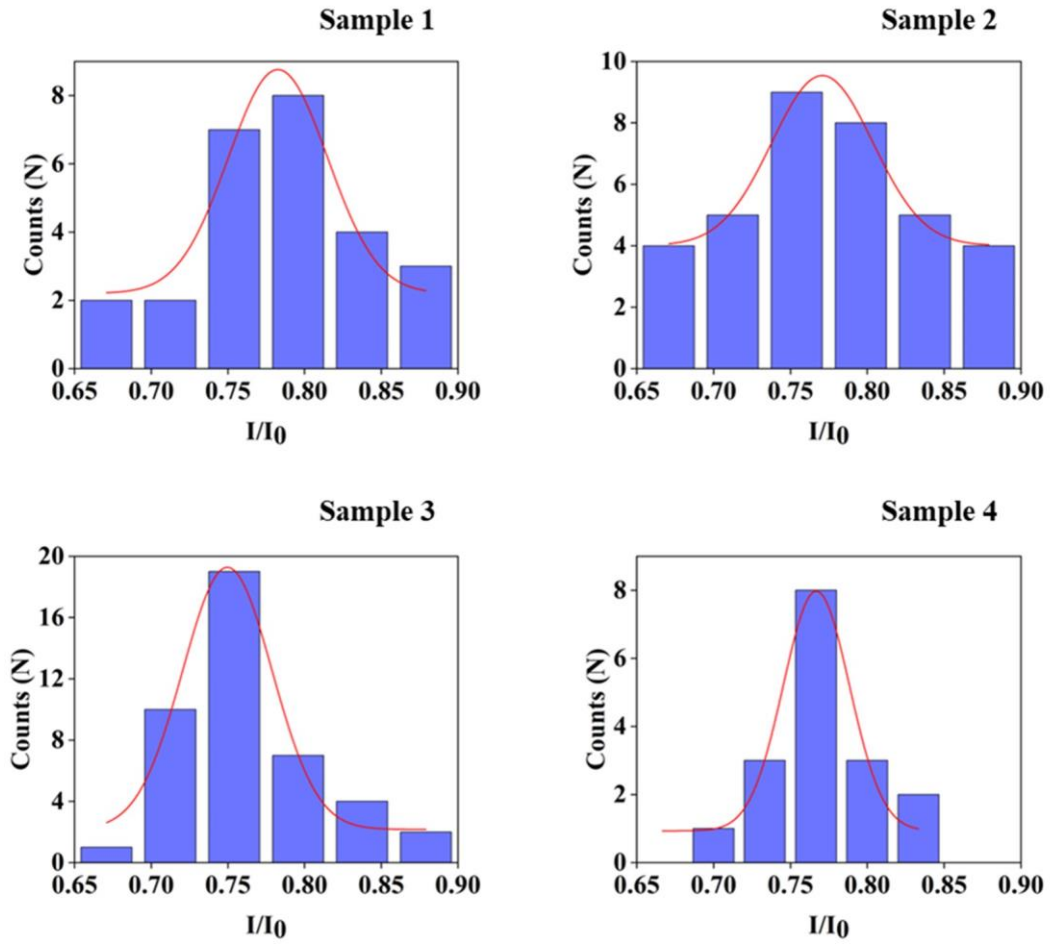


Figure S11. I/I_0 histogram of the events produced by the 4 positive field samples.

Field sample 1

Download GenBank Graphics				
Ralstonia solanacearum strain FJAT1303.F1 chromosome, complete genome				
Sequence ID: CP052128.1 Length: 3649900 Number of Matches: 1				
Range 1: 1185402 to 1185566 GenBank Graphics ▼ Next Match ▲ Previous Match				
Score	Expect	Identities	Gaps	Strand
287 bits(155)	4e-73	162/165(98%)	2/165(1%)	Plus/Minus
Query 3	ATC-GCGTCCAGGCGACCTACATCGACGAACGAACGAAGTGGCGAACGGGCTTTCCCAAC	61		
Sbjct 1185566	ATCAGCGCCCAGGCGACCTACATCGACGAACGAACGAAGTGGCGAACGGGCTTTCCCAAC	1185507		
Query 62	GGAAGCAATGACAACCCACGTCCAAGCTGCGCTCTTTCAGGGCAAAGGCAACCTACGTG	121		
Sbjct 1185506	GGAAGCAATGACAACCCACGTCCAAGCTGCGCTCTTTCAGGGCAAAGGCAACCTACGTG	1185447		
Query 122	TACGACCACACCTACGGCGTGACCGCTGGCGT-TTCAAGGTGACC	165		
Sbjct 1185446	TACGACCACACCTACGGCGTGACCGCTGGCGTCTTCAAGGTGACC	1185402		

Field sample 2

Download GenBank Graphics				
Ralstonia solanacearum strain FJAT1303.F1 chromosome, complete genome				
Sequence ID: CP052128.1 Length: 3649900 Number of Matches: 1				
Range 1: 1185402 to 1185562 GenBank Graphics ▼ Next Match ▲ Previous Match				
Score	Expect	Identities	Gaps	Strand
285 bits(154)	1e-72	159/161(99%)	1/161(0%)	Plus/Minus
Query 3	GCGTCCAGGCGACCTACATCGACGAACGAACGAAGTGGCGAACGGGCTTTCCCAACGGAA	62		
Sbjct 1185562	GCGCCCAGGCGACCTACATCGACGAACGAACGAAGTGGCGAACGGGCTTTCCCAACGGAA	1185503		
Query 63	GCAATGACAACCCACGTCCAAGCTGCGCTCTTTCAGGGCAAAGGCAACCTACGTGTACG	122		
Sbjct 1185502	GCAATGACAACCCACGTCCAAGCTGCGCTCTTTCAGGGCAAAGGCAACCTACGTGTACG	1185443		
Query 123	ACCACACCTACGGCGTGACCGCTGGCGT-TTCAAGGTGACC	162		
Sbjct 1185442	ACCACACCTACGGCGTGACCGCTGGCGTCTTCAAGGTGACC	1185402		

Field sample 3

Download GenBank Graphics				
Ralstonia solanacearum strain FJAT1303.F1 chromosome, complete genome				
Sequence ID: CP052128.1 Length: 3649900 Number of Matches: 1				
Range 1: 1185402 to 1185562 GenBank Graphics			▼ Next Match ▲ Previous Match	
Score	Expect	Identities	Gaps	Strand
285 bits(154)	1e-72	159/161(99%)	2/161(1%)	Plus/Minus
Query 7	GCG-CCAGGCGACCTACATCGACGAACGAACGAAGTGGCGAACGGGCTTCCCAACGGAA	65		
Sbjct 1185562	GCGCCAGGCGACCTACATCGACGAACGAACGAAGTGGCGAACGGGCTTCCCAACGGAA	1185503		
Query 66	GCAATGACAACCCACGTCCTCAAGCTGCGCTCTTTCAGGGCAAAGGCAACCTACGTGTACG	125		
Sbjct 1185502	GCAATGACAACCCACGTCCTCAAGCTGCGCTCTTTCAGGGCAAAGGCAACCTACGTGTACG	1185443		
Query 126	ACCACACCTACGGCGTGACCGCTGGCGT-TTCAAGGTGACC	165		
Sbjct 1185442	ACCACACCTACGGCGTGACCGCTGGCGTCTTCAAGGTGACC	1185402		

Field sample 4

Download GenBank Graphics				
Ralstonia solanacearum strain FJAT1303.F1 chromosome, complete genome				
Sequence ID: CP052128.1 Length: 3649900 Number of Matches: 1				
Range 1: 1185402 to 1185557 GenBank Graphics			▼ Next Match ▲ Previous Match	
Score	Expect	Identities	Gaps	Strand
281 bits(152)	2e-71	155/156(99%)	1/156(0%)	Plus/Minus
Query 9	CAGGCGACCTACATCGACGAACGAACGAAGTGGCGAACGGGCTTCCCAACGGAAGCAAT	68		
Sbjct 1185557	CAGGCGACCTACATCGACGAACGAACGAAGTGGCGAACGGGCTTCCCAACGGAAGCAAT	1185498		
Query 69	GACAACCCACGTCCTCAAGCTGCGCTCTTTCAGGGCAAAGGCAACCTACGTGTACGACCAC	128		
Sbjct 1185497	GACAACCCACGTCCTCAAGCTGCGCTCTTTCAGGGCAAAGGCAACCTACGTGTACGACCAC	1185438		
Query 129	ACCTACGGCGTGACCGCTGGCGT-TTCAAGGTGACC	163		
Sbjct 1185437	ACCTACGGCGTGACCGCTGGCGTCTTCAAGGTGACC	1185402		

Figure S12. DNA sequencing of the 4 positive field sample. Alignment of DNA sequencing results of the 4 positive field samples by Basic Local Alignment Search Tool in NCBI website.

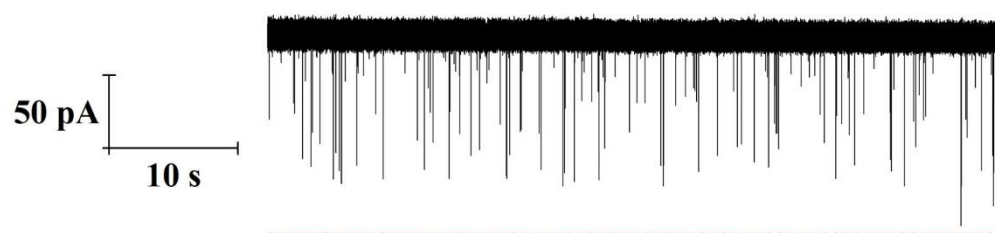


Figure S13. Current trace of the negative field sample (sample 5).

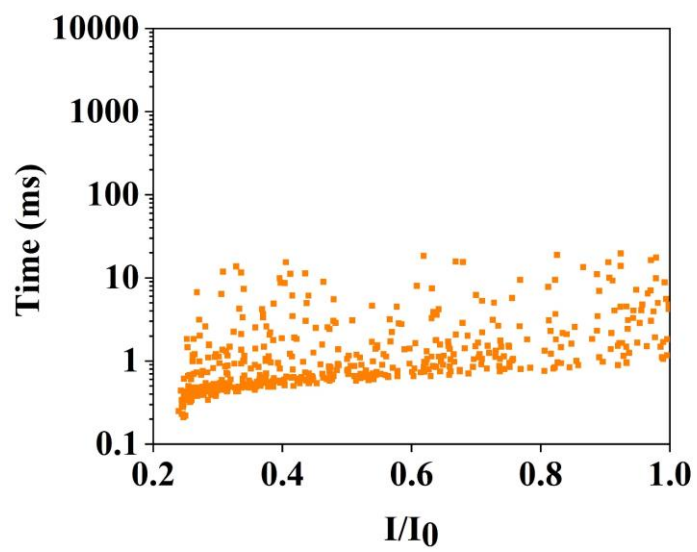


Figure S14. Scatter plot of the sample 5 (n=524).