Supplementary information

Experimental Section

Sampling inoculated plants

Pathogen-inoculated *Arabidopsis thaliana* were sampled at various times after infection and scored (S1 to S5) based on their severity of symptom development.[1] For *Pseudomonas syringae*, S1: 1 day, S2: 2-3 days, S3: 4-5 days, S4: 6-7 days, S5: 8-9 days. For *F. oxysporum* f. sp. *conglutinans*, S1: 3-6 days, S2: 7-10 days, S3: 11-15 days, S4: 16-20 days, S5: 21-25 days. For *Botrytis cinerea,* S1: 2-4 days, S2: 5-7 days, S3: 8-10 days, S4: 11-13 days, S5: 14-16 days.

Nucleic acid extraction

Plant genomic DNA was extracted from a single Arabidopsis leaf (~300 mg) using an optimized lysis buffer (50 mM Tris-HCl pH 8.0, 1.5 M guanidium-HCl, 2% w/v PVP40 and 1% v/v Triton-X). For DNA applications, 400 ng/µL RNase A was added to the lysis buffer but not for RNA applications. Plant tissue was macerated in a 1.5 mL tube with a disposable plastic pestle in the presence of 200 µL of lysis buffer. After 10 min incubation at room temperature, the lysate was cleared using a homemade filtration device made from a common filtered pipette tip. Using this approach further enhanced the potential for low resource applications. For non-plant applications, 4 volumes of lysis buffer without PVP40 was used with every volume of sample. Nucleic acids were then purified using a modified SPRI protocol[2, 3]. Briefly, a single drop (~10 µL) of the cleared lysate was incubated with 1.8 volumes of 1 micron carboxylic acid coated magnetic beads (Thermo Fisher, Cat# 4515-2105-050250) in a binding buffer (10 mM Tris-HCl pH 8.0, 20% PEG8000, 2.5 M NaCl) for 5 mins. DNA bound beads were then separated from the lysate with a magnet and washed twice with 100% isopropanol, two 80% ethanol washes and eluted in one drop of water (~10 µL). All chemicals were purchased from Sigma Aldrich unless stated otherwise.

Nucleic acid amplification

The TwistAmp Basic RPA Kit (TwistDX, cat# TABAS01kit) was used as recommended by the manufacturer with some modifications. Briefly 12.5 µL reactions were performed at 37°C for 30mins using 1 µL of the nucleic acid extraction and 480-600 nM of each primer (Table S1). For RNA applications, 50 units of MMuLV reverse transcriptase (New England Biolabs, cat# M0253S) were added to the RPA reaction. Following amplification, 5 µL of the RPA reaction was verified by gel electrophoresis.

Bridging Flocculation Assay

5 µL of amplified product was used in the flocculation assay by incubating with 1.5 - 1.8 volumes of SPRI bead solution for 5 minutes. After bead separation with a magnet and an 80% ethanol wash, 30 µL of flocculation buffer (100mM sodium acetate, pH 4.4, 1% v/v Tween20) was added to the beads and gently agitated.

Target/ GenBank Accession	5'-Forward-3'	5'-Reverse-3'
F.oxysporum* f.sp. *conglutinans AGNF01000001.1	GCTCTTGATTTAGGTACAACTCTTTCCCTCGTC	ATATATCTGTATAGGAATCCCACTGAATTTTTC
Botrytis cinerea ALOC01000004.1	TTTCCACAGGGTTTGTGTACGAGATTGGTATTC	TTCTCCGGTGTCCGTTCGCACTGTAGACAATCG
Pseudomonas syringae AE016853.1	TTTGTCCGAAACGACGTACAGCCATTTAACCTT	TTCTACGTCGGGGTATTTACTAGCTGGAAAAG
F.oxysporum cubense AMGP01000029.1	ATTGAAGGACTCATACAAGGTTGCATCAAAATA	TTTCCTTTTGCAACTCCTACAGAGTGTCTATAA
Cucumber mosaic virus RNA3 Coat AJ585517.1	AGTTAATCCTTTGCCGAAATTTGATTCTAC	GTGCTCGATGTCAACATGAAGTACTAGCTC
Bovine HPV TK1 NC_001847.1	GGAAGATCTGCTCATGCTCGCGGCCGCCATGCC	GAGCGCGTAAGCATTGCGCACAGCGACCAGAAA
Bovine HPV Glycoprotein B NC_001847.1	AAGTGGCGCGAGGCGGACGAAATGCTGCGAGAC	ACGTGCGTGCCGTTGTAGCGCTCGCGGTAGACG
***E.coli uidA* gene** NC_017635.1	CTGTGACGCACAGTTCATAGAGATAACCTTC	AAAAGCAGTCTTACTTCCATGATTTCTTTAACT
HIV M19921.2	AAATTAACAATTACACAAGCTTAATACACTCC	TATAGAAAGTACAGCAAAAACTATTCTTAAACC
Plasmodium Falsiparum MSP1 XM_001352134.1	TTGAAGGAAGTAAGAAAACAATTGATCAAAATA	CTAAAACGCTTATTAAATTATGTGCTTCTTCTA
Tuberculosis CFP10 CP003248.2	ATTTTGGCGAGGAAGGTAAAGAGAGAAAGTAGT	GAGTTCCTGCTTCTGCTTATTGGCTGCTTCTT
Tuberculosis ESAT-6 CP003248.2	CAATCCAGGGAAATGTCACGTCCATTCATTCC	CCTATGCGAACATCCCAGTGACGTTGCCTTC
Influenza A H1N1 CY058490.1	CCATTAATAAGACATGAGAACAGAATGGTTC	AAATTTTCAAGGAGATCATTTTTCAGACCAGTG

Table S1. List of RPA primers used. GenBank Accession numbers are as given. All primers were purchased from Integrated DNA Technologies (IDT).

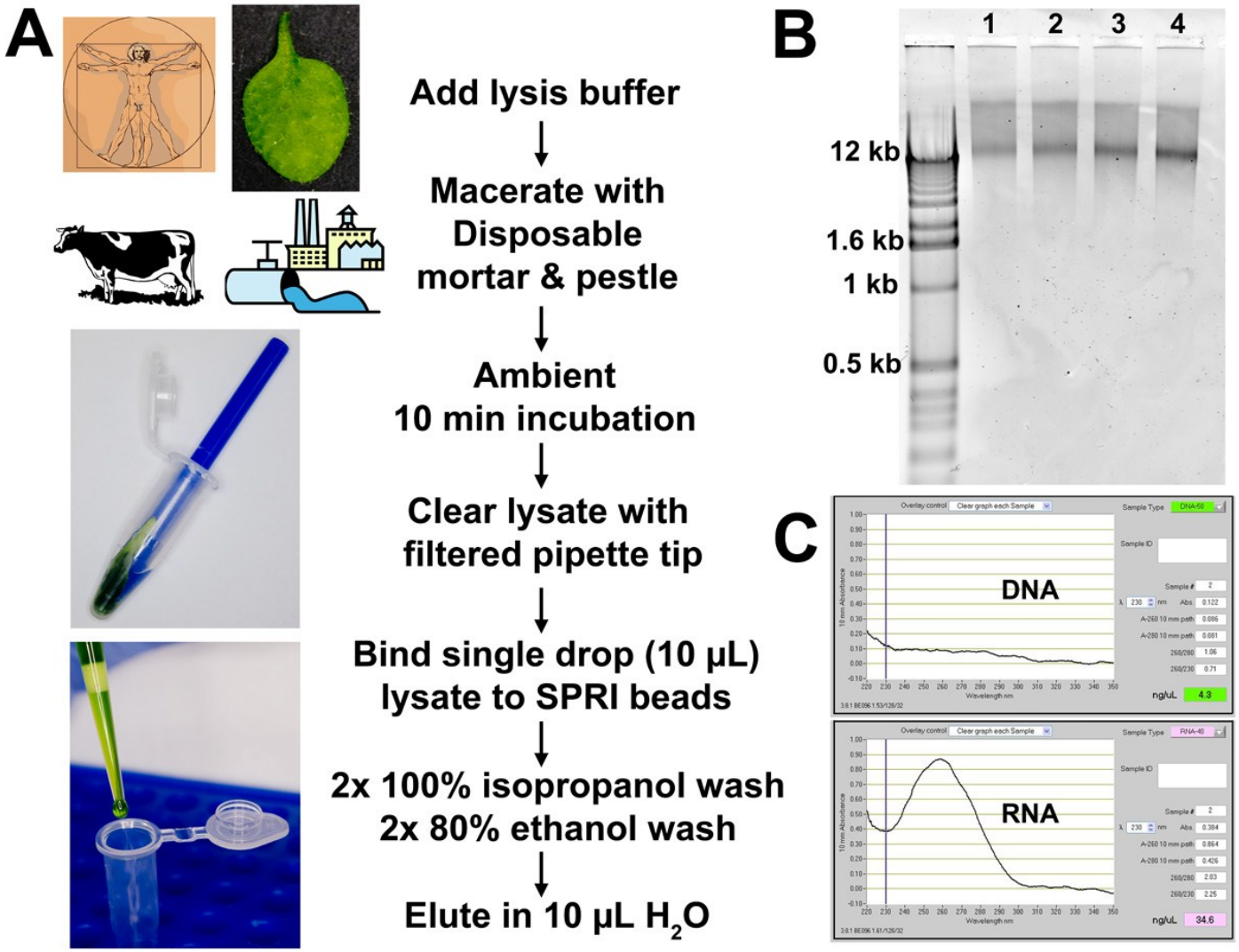

Figure S1. Precision nucleic acid extraction protocol. (A) Graphical representation of the extraction protocol. Photographs show the typical leaf used for extraction, manual maceration of the leaf using a disposable mortar and pestle and clearing the lysate of cellular debris using a common pipette tip. (B) Gel electrophoresis of four independently extracted DNA samples, all 10 µL elute was used. The high molecular weight suggests the good integrity of the extracted DNA. (C) Spectrometry analysis of extracted nucleic acids. Top: DNA, Bottom: RNA.

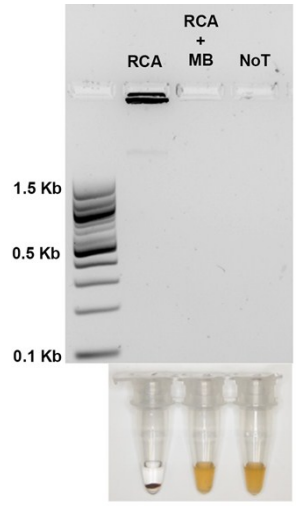

Figure S2. Using the flocculation assay to detect long single stranded DNA generated by rolling circle amplification (RCA). Mung bean nuclease (MB), a single stranded DNA exonuclease, was used to demonstrate that RCA generated DNA was indeed single stranded. As expected, flocculation occurred only when long single stranded DNA was present but not in MB digested and no template (NoT) controls. Circle sequence: *TGGTCTTAAAAACTCTTTCGTTGTCATTGGGATAGGCGATTCTAAATTTCTCAACGAAATCTGG was purchased from IDT with a 5'phosphate modification and circularized using the CircLigase II ssDNA Ligase Kit (Epicentre, Cat#* CL9021K) following the manufacturer's recommendation. *The Primer sequence:* AGAATCGCCTATCCCAATGACA was used to trigger RCA.

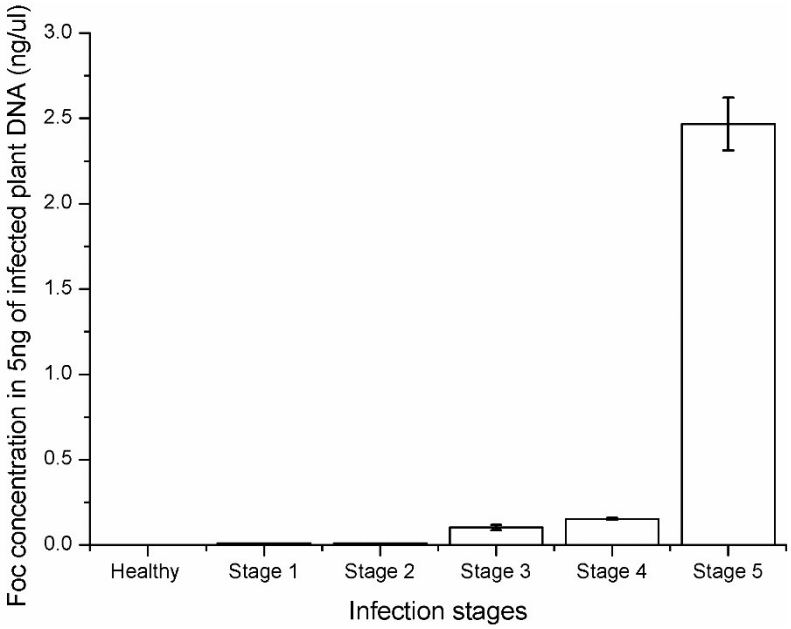

Figure S3. qPCR quantification of *F.oxysporum conglutinans* (FOC) in 5 ng of extracted DNA from leaves at various stages of infection. The amount of pathogen DNA was estimated from calibration plot of known target concentrations. Pathogen DNA was detectable from stage 3 infection onwards using the same primers for RPA.

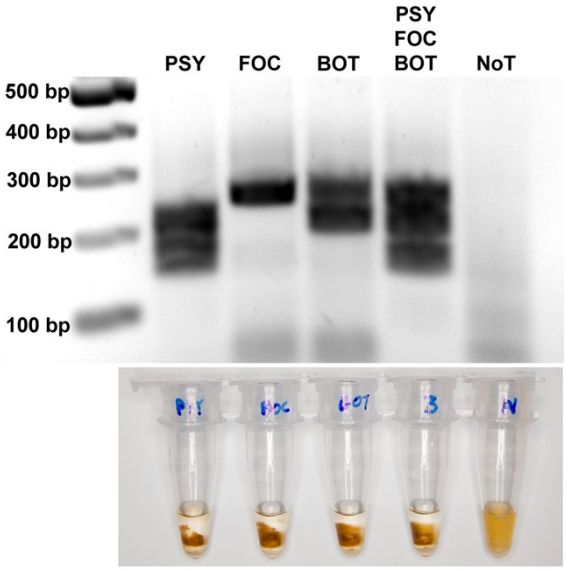

Figure S4. Testing the 3-primer mix for simultaneous detection of *Pseudomonas syringae* (PSY), *F. oxysporum* f.sp. *conglutinans* (FOC) and *Botrytis cinerea* (BOT) controlled with no template control (NoT). Primers used were the same ones used in Fig 3 of the main text. 500 nM of primers were used at a 1:1:1 ratio. Purified DNA from the respective pathogens were used in this experiment. For individual pathogen amplifications, 10 pg of PSY, 100 pg of FOC and BOT were used. For the 3 pathogen detection, 3.33 pg PSY, 33.3 pg of FOC and BOT were used. While non-specific bands were seen for FOC and BOT targets, most likely a result of cross-reactivity between primers and pathogens, this was viewed as advantageous for our flocculation readout. This was because larger amounts of high molecular weight amplicons were generated and this in turn, facilitated DNA mediated bridging flocculation, thus potentially increasing sensitivity. Since no amplification and flocculation were observed in the NoT control, we were confident that the assay was still specific only to the presence of any one of the pathogens and could now be used with infected leave extracts.

Methods	Detection limit	Detection time (From sampling to detection)	References
Flocculation Assay	Less than 0.3 pg/ µL	90 mins	This study
Koch's postulate	-	~20 days	Opgenorth, 1983[4]
ELISA	0.01 µg of antigen	~ 1.5 day	Fogliano, *et. al.* 1999[5]
Immunofluorescence colony staining	70 cfu/L	~3days	Riffaud, and Morris 2002[6]
Dot blot hybridization	1×10^2 cfu	~1.5day	Fanelli, *et. al*.2007[7]
Real time PCR	1pg of gDNA	~ 3 hrs	Green, *et. al.* 2009[8]
PCR (gDNA)	Not stated	~ 6 hrs	Schmidt, *et. al.* 2009[9]
PCR (cell)	1.41×10^3copies/µL	~ 2 hrs	Choi, *et. al.* 2013[10]
PCR/RFLP	102 CFU/mL	~ 3 days	Biondi, *et. al.* 2013[11]
Gold nanoparticle probe	15 ng/µL	~ 2.5 hrs	Vaseghia, *et. al.* 2013[12]
Selective medium (seed)	0.08% contamination	~ 7 days	Suzuki, *et. al.* 2014[13]

Table S2. A brief comparison of various methods detecting Pseudomonas syringae in literature

References

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