Supporting Information

Rapid naked-eye detection of grapevine red-blotch viral infection using a plasmonic CRISPR Cas12a assay

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1. Materials. Solutions of 20-nm gold nanoparticles (AuNPs), TWEEN 20, 10 × phosphate buffer saline (PBS), magnesium chloride ($MgCl_2$), and sodium chloride (NaCl) were purchased from Sigma (Oakville, ON, Canada). EnGen Lba Cas12a (Cpf1), WarmStart LAMP Kit, OneTaq Master Mix, and 10 × NEBuffer™ 2.1 Buffer were purchased from New England Biolabs Ltd. (Whitby, ON, Canada). NANOpure H2O (> 18.0 MΩ), purified using an Ultrapure Mili-Q water system, was used for all experiments. All DNA samples and the guide RNAs were purchased from Integrated DNA Technologies (Coralville, IA) and purified using high-performance liquid chromatography. The DNA sequences and modifications are outlined in Table S1.

Table S1.DNA sequence and modifications.

DNA name	Sequence (5'-to-3')
1.AuNP-immobilized Arm DNA sequence for A-AuNPs & B-AuNPs and Linker	
Arm-A	HS-AAA AAA AAA ACC TCA CCA CCA ACA C
Arm-B	HS-AAA AAA AAA ACA CAC ACA CTC ACA C
Linker	GTG TGA GTG TGT GTG GTG TTG GTG GTG AGG
2.Random Targets Detection	
NTS-35	GCT TGT GGC CG **TTTA** CGT CGC CGT CCA GCT CGA CC
TS-35	GGT CGA GCT GGA CGG CGA CG **TAAA** CGG CCA CAA GC
crRNA	UAA UUU CUA CUA AGU GUA GAU CGT CGC CGT CCA GCT CGA CC
3.Grapevine Red Blotch-associated Virus Detection after PCR Amplification	
NTS_{GRBV}	GCA CGT CGG CGA CAT CTC TGG GCT TTG TGA TAT TGG GGT GAT AGG AAT GAG ACG ATA TGG TGA TGT CAA AGT GT **TTTG** GAT TGC GAA TAG CCT GTC GT T TTT TGA AGA TGA GCA AGG CGT GTA GGT GTG G
TS_{GRBV}	C CAC ACC TAC ACG CCT TGC TCA TCT TCA AAA AAC GAC AGG CTA TTC GCA ATC **CAAA** AC ACT TTG ACA TCA CCA TAT CGT CTC ATT CCT ATC ACC CCA ATA TCA CAA AGC CCA GAG ATG TCG CCG ACG TGC
Primer-D	GCA CGT CGG YGA CAT CTC TG
Primer-R	CCA CAC CTA CAC GCC TTG C
$crRNA_{GRBV}$	UAA UUU CUA CUA AGU GUA GAU GAT TGC GAA TAG CCT GTC GT

* For all dsDNA target sequence, bolded region is PAM sequence (TTTN).

* For all dsDNA target sequence, underlined region is the sequence detected by crRNA-Cas12a complexes.

2. Preparation of DNA functionalized AuNPs. DNA functionalized AuNPs were prepared by conjugating thiolated DNA oligonucleotides onto the 20nm AuNPs according to our previously established protocol.[1] Briefly, 40 µL of 5 µM DNA-A or DNA-B were mixed with 500 µL of 20nm AuNPs (1.16 nM), respectively. The mixture was incubated at room temperature for 12hrs and slowly mixed with 16.5 µL of 3 M NaCl solution, followed by 10s of sonication. The salt aging process was repeated five times with 1h interval. The mixture was then centrifuged at 13,500 rpm for 30 min to separate the DNA-AuNPs from excess thiolated DNA oligonucleotides. The supernatant was discarded and DNA-AuNPs were washed with 1 mL 1×PBS buffer (ph 7.4) containing 0.01% TWEEN 20. The washing steps were repeated for four times. The DNA-AuNPs was finally dispersed in PBS buffer at 2 nM final concentration and stored at 4°C.

3. Plasmonic assay protocol. For a typical plasmonic assay, a reaction solution containing 1 nM AuNP-A, 1 nM AuNP-B and 30 nM *S* or Cas12a degraded *S* was incubated at 50°C for 15 minutes and then cooled to the room temperature. The color of the solution was recorded as an image and the absorbance of which was then scanned from 450 nm to 700 nm at a resolution of 1 nm using a Multimode Microplate reader (SpectraMax i3, Molecular Devices).

4. Plasmonic CRISPR-Cas12a assay. For a typical plasmonic CRISPR-Cas12a assays, a reaction mixture containing 30 nM Cas12a, 30 nM of gRNA, 60 nM *S*, and varying concentrations of target DNA, PCR amplicon, or LAMP amplicon was incubated at 37°C for 30 min. followed by an enzyme denaturation step. 10 µL of this reaction mixture was then mixed with an equal volume of AuNP solution and then tested using the plasmonic assay protocol outlined above.

5. PCR protocol. For a typical PCR reaction, 2 µL target DNA was added to a reaction mixture containing Taq 2× Master Mix, 200 nM reverse primer, 200 nM forward primer to a final volume of 25 µL. PCR was then performed for 35 cycles in a BioRad T100 Thermal Cycler.

6. Polyacrylamide gel electrophoresis (PAGE). A 5-µL solution containing PCR or LAMP amplicons was mixed with loading buffer and then loaded onto 6% PAGE gel. A voltage of 110 V was applied for driving the electrophoresis. After electrophoresis, the gel was stained with Ethidium Bromide and imaged using Gel Doc XR+ Imager System (BioRad).

7. Grapevine sample collection and DNA extraction. Field grapevine samples representing both white- (Vidal Blanc) and red-fruited (Baco Noir) interspecific hybrids as well as white- (Chardonnay and Riesling) and red-fruited (Cabernet Franc and Cabernet Sauvignon) *V. vinifera* cvs. were collected from commercial vineyard blocks in the Niagara region during the month of September in 2018. Total nucleic acids (TNAs) were extracted from individual petiole samples (four petioles/vine) using a previously reported method.[3,4] Briefly, samples extracts were prepared using 250 mg of petiole tissue with the aid of semi-automated HOMEX 6 homogenizer (BIOREBA AG, Switzerland) in 5 ml of extraction buffer and to isolate TNAs, 4µl of extraction buffer was added into 25µl of extraction solution with 1% 2-mercaptoethanol and denatured at 95°C for 10 min before using as template for end-point and ddPCR assays. TNAs isolated from healthy and infected leaf material from grapevines maintained at the phytotron facilities at Brock University were used as negative and positive controls, respectively.

8. Grapevine sample testing using droplet digital PCR (ddPCR) and end-point PCR. Each ddPCR reaction was prepared in a total volume of 20 µL, which consisted of 10 µL of 2 × QX200™ ddPCR™ EvaGreen Supermix (Bio-Rad, Mississauga, ON, Canada), 100nM each of 706D-F (5'-GCACGTCGGYGACATCTCTG-3') and 706D-R (5'-CCACACCTACACGCCTTGC-3') primers, 8µL RNase/DNase-free water and 1µL template DNA (30 ng/ µL). The ddPCR reaction mixture was added to 70 µL of QX200™ Droplet Generation Oil for EvaGreen (Bio-Rad, Mississauga, ON, Canada).

Droplets were generated using QX200™ AutoDG™ Droplet Digital™ PCR System (Bio-Rad, Mississauga, ON, Canada). The PCR consisted of initial enzyme activation step at 95°C for 5 min and then 40 cycles of denaturation at 95°C for 30 sec and annealing and extension at 60°C for 1 min, followed by signal stabilization at 4°C for 5 min and 90°C for 5 min. Ramp rate of 2°C per second was followed during enzyme activation and denaturation steps. Three technical replicates were performed for all six samples tested as well as controls. The end-point PCR was performed in a C1000 Touch Thermal Cycler (Bio-Rad, Mississauga, ON, Canada) using primer sets (GVGF1 5' CTCGTCGCATTTGTAAGA-3') and GVGR1 (5-ACTGACAAGGCC TACTACG-3') that were specific to partial ORF of V1 and V2 genes (256 to 821) of GRBV.[4]

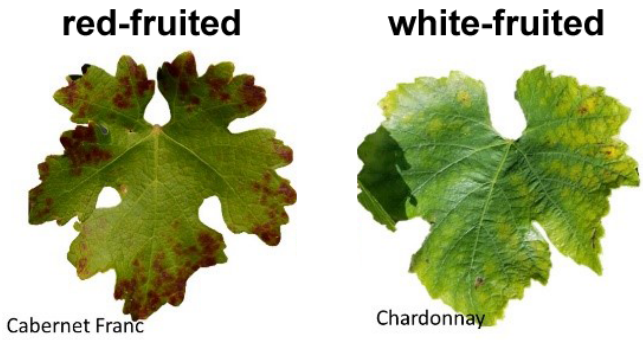

Figure S1. Representative symptoms of grapevine red blotch virus infections on the leaves of red-fruited (Cabernet Franc, left) and white-fruited (Chardonnay, right) *Vitis Vinifera*. The reddish purple patches on the leaves coalesce into bloches covering the entire leaf surface in case of red-berried cultivars, Cabernet Franc (left), whereas on white fruited cultivar, Chardonnay, the symptoms are less apparent with yellowish discoloration of leaf edges and necrotic spots can be seen.

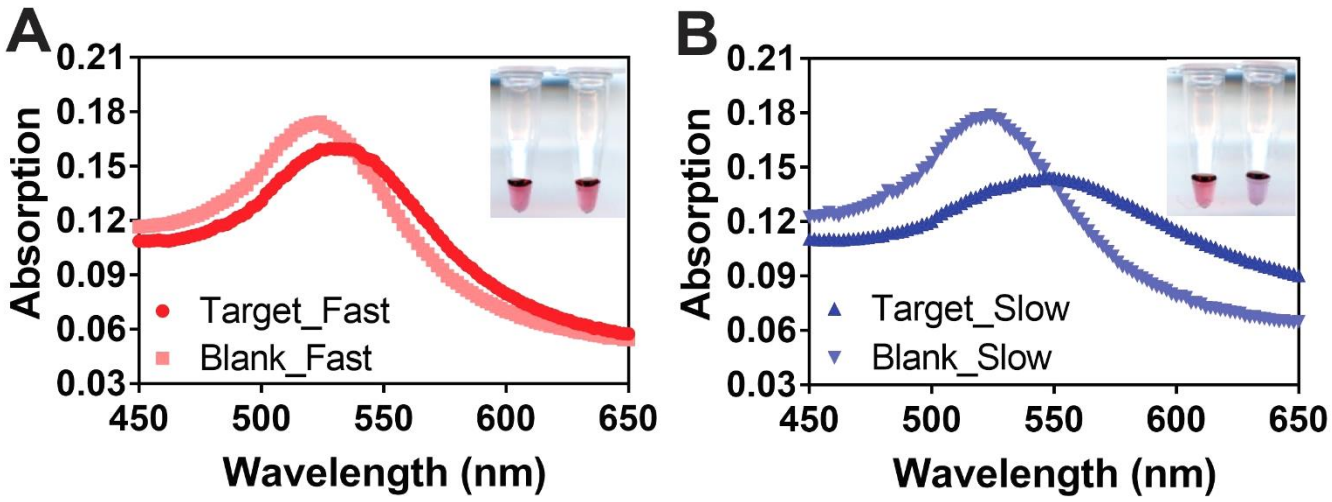

Figure S2. **Optimal condition for the plasmonic assay in the presence of CRISPR Cas12a.** The experiments were performed to confirm that the extended reaction time from 5 min (fast reaction, **A**) to 15 min (slow reaction, **B**) could facilitate the crosslinking of DNA-AuNPs in the presence of CRISPR Cas12a reagents. The wavelength shift of the maximal absorbance was increased from 4 nm (**A**) to 32 nm (**B**).

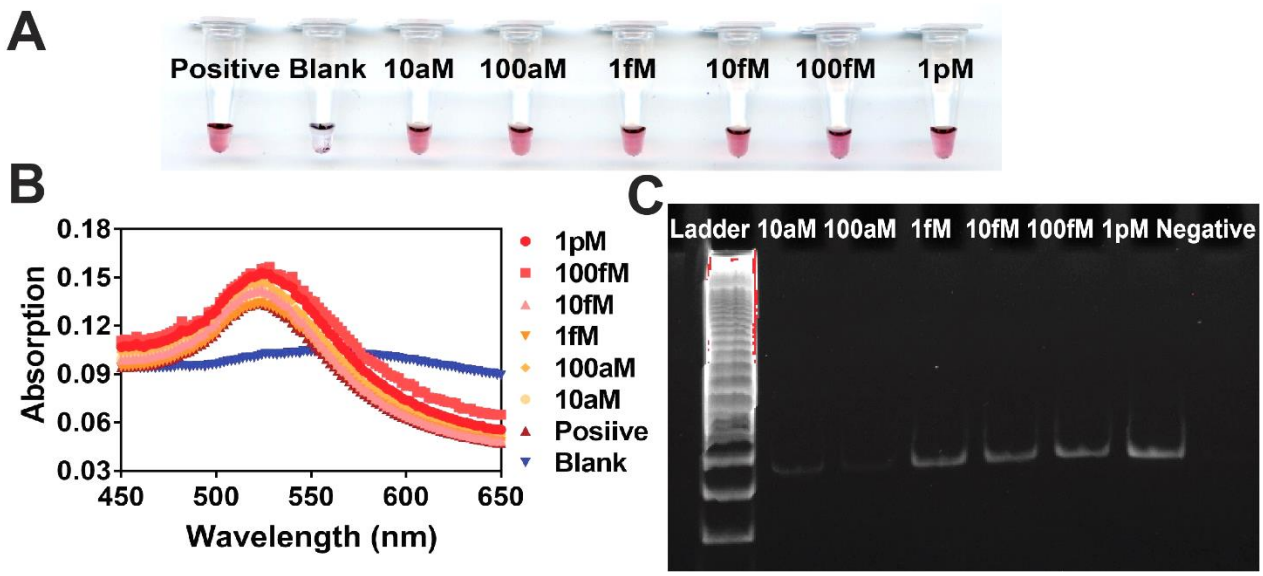

Figure S3. **(A)** Visual analysis of PCR amplicons of varying concentrations of synthetic DNA standards using the plasmonic CRISPR Cas12a assay. (**B**) Absorbance spectra of the colorimetric readout on the same samples shown in (**A**). (**C**) Parallel analyses of PCR amplicons using PAGE.

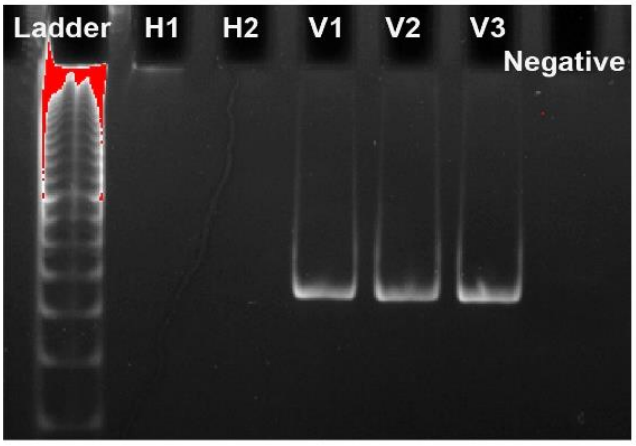

Figure S4. PAGE analysis of PCR amplicons of GRBV infected grape samples (V1, V2, V3) and health grape controls (H1, H2).

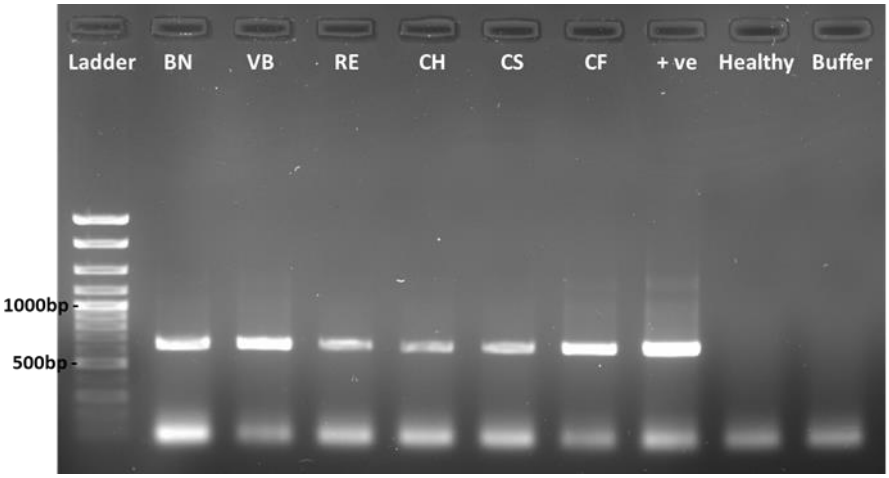

Figure S5. Detection of GRBV isolates by end-point PCR and electrophoresis. Gel Green (Biotium, USA) stained 1% agarose gel electrophoresis image of the PCR amplicons of grapevine red blotch virus (GRBV). Names of the 6 isolates along with controls were mentioned on the top of the gel in white and the size of the reference marker mentioned on the left in black. BN-Baco Naoir; VB-Vidal Blac; RE-Riesling; CH-Chardonnay; CS- Cabernet Sauvignon; CF- Cabernet Franc.

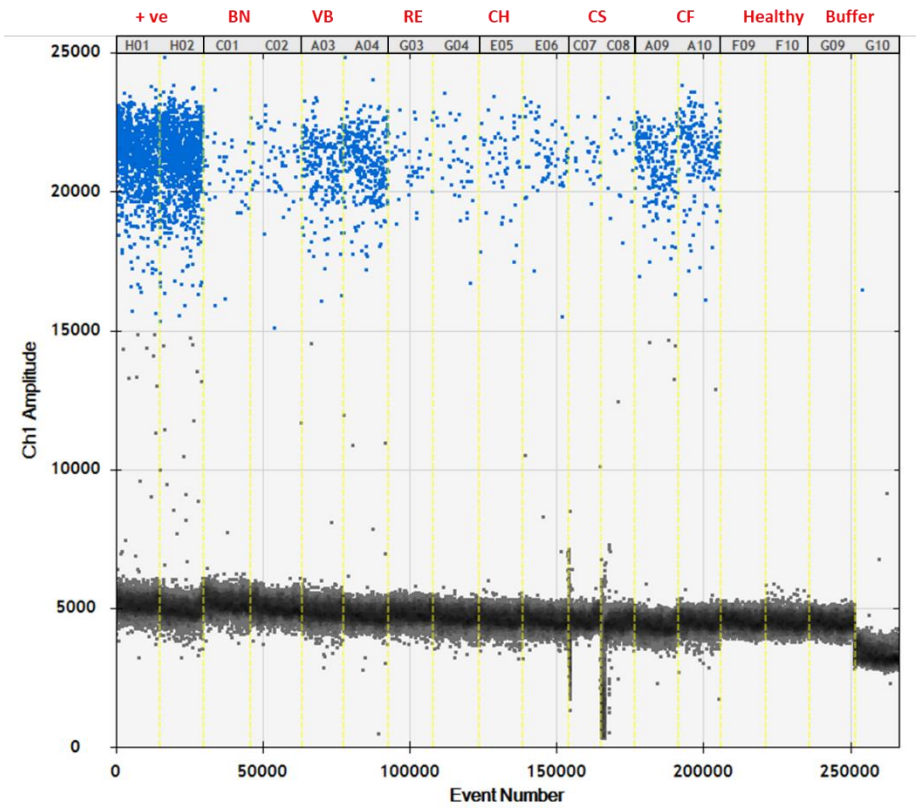

Figure S6. Detection of GRBV isolates by ddPCR. Fluorescence amplitude plot representing the absolute quantification of grapevine red blotch virus (GRBV) DNA from 6 different isolates. Droplets in blue on top represents positive amplicons and the grey droplets on the bottom represents no GRBV amplificaiton. The PCR reactions for each isolate along with positive, healthy and buffer controls are carriedout in duplicates and are represented by vertical dotted yellow line. BN-Baco Naoir; VB-Vidal Blac; RE-Riesling; CH-Chardonnay; CS- Cabernet Sauvignon; CF- Cabernet Franc.

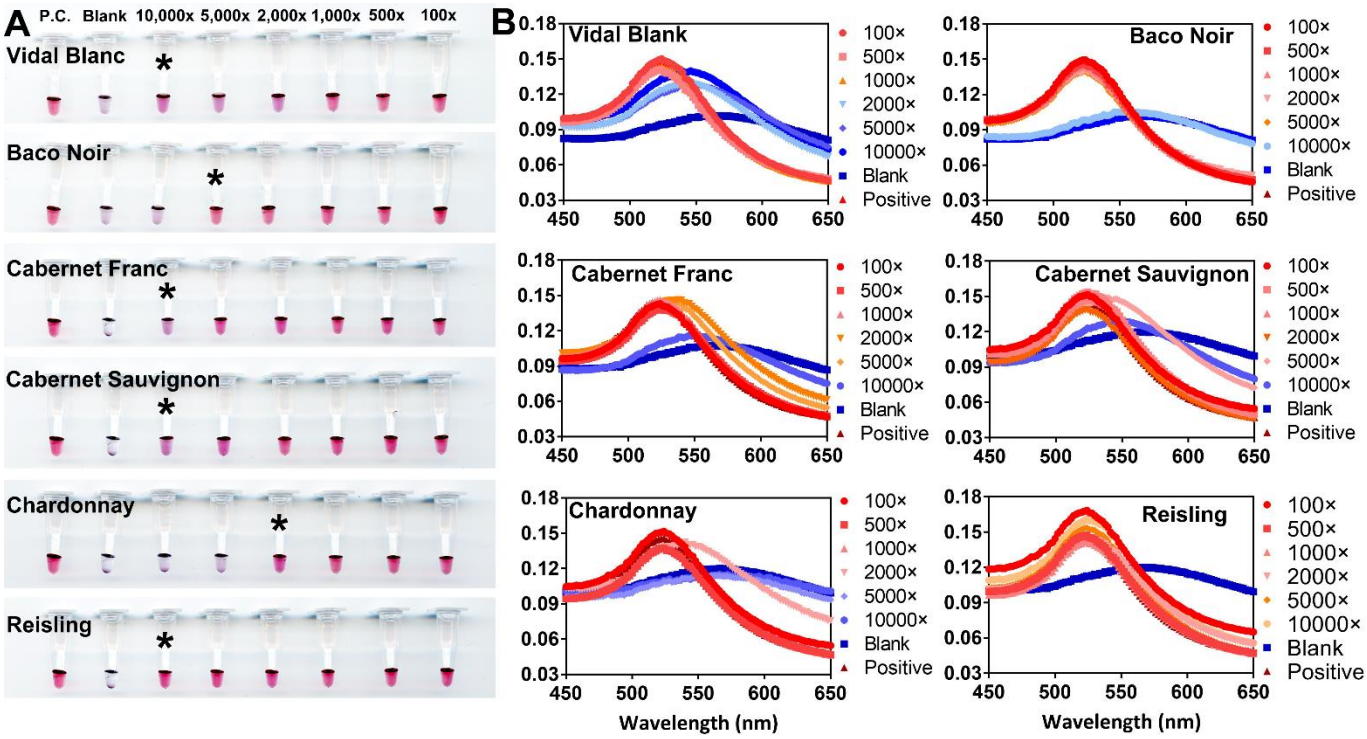

Figure S7. (**A**) Visual detection of GRBV infected grapevine leaf samples with varying dilution factors using PCR and plasmonic CRISPR Cas12a assay. (**B**) Absorbance spectra of the colorimetric readout on the same samples shown in (**A**).

References

[1] Y. Li, G. A. Wang, S. D. Mason, X. Yang, Z. Yu, Y. Tang, F. Li, *Chem. Sci.* **2018**, *9*, 6434-6439.

[2] H. Wang, S. Kim, W.S. Ryu, *J. Virol*. **2009,** 83, 5815- 5824.

[3] A. Rowhani, L. Biardi, R. Johnson, P. Saldarelli, Y. P. Zhang, J. Chin, M. Green. Simplified sample preparation method and one-tube RT-PCR for grapevine viruses. Extended Abstracts of the 13th Meeting of the International Council for the Study of Virus-like Diseases of the Grapevine, Adelaide, Australia. **2000**. 82.

[4] M. Al Rwahnih, A. Dave, MM, Anderson, A. Rowhani, JK. Uyemoto, MR. Sudarshana. *Phytopathology* **2013**, *103*, 1069–1076