

SERS combined with PCR as a potent tool for detecting mutations: A case study of tomato plants

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Supporting Information

RNA Silencing:

RNA silencing, in plants, utilizes the help of Dicer-like proteins (DCL). During this process, a small molecule of RNA is produced (sRNA) which binds with the DCL proteins to form the RNA-induced silencing complex (RISC). This complex is responsible for the inhibition of viral protein formation by interacting with the viral messenger RNA (mRNA).¹ In the case of TYLCV, plants carrying the genes required for resistance, if challenged by the virus show low levels of viral replication and spread of infection with moderate or no visual symptoms.² The genes responsible for this are the Ty1 and Ty-3 genes. These are alleles of the same gene and code for RNA-dependent RNA polymerases from a class of functionally unknown RDR genes, which are thought to be involved with the RNA silencing.³

Materials and Methods:

1X Phosphate Buffer Saline:

137 mM NaCl, 2.7 mM KCl, 4.3 mM Na₂HPO₄, 1.47 mM KH₂PO₄

Mastermix:

This Master Mix consists of AmpliTaq Gold™ DNA Polymerase UP (Ultra-Pure), dNTPs, MgCl₂, and other essential components necessary for PCR.

Sequence definitions for PCR-SERS:

Tomato Indel sequence for Ty-3 gene

Indel marker: **With insertion** **CCTTCTTGTATA** (Resistant type)

TTTATATAAGGAAATAATAAATTCAATATGAAAAAGATTTACATTTGACCAGAAA
TAGTTCAAATAATCAATACTTTAATTTTTTTAAGGAAAAAATAAATTTAAATAAA
GTAAAAAATCAAGACAATACCTAAAAATATATCTACATTTTTCAAATTAATAAA
TAATAATTTAAATATAAAATATATGAAAAACAATTTAAACATGTTTTTGACAAGA
TTCCCTTAAACCTATCCTCAGTGTTTCGGTCAAAAAGTATGACTTCACTTGTACGG
CATTTAACATGCAATAGTGTAGTTACTACTACCGCACTAGTTCTGTTTTCTCCCA
AAACTCATTATTTAATTTTTCTTCTTTAACAAAGTTGACGAGACAAAAGCAAAAGT
ATGAAGAATTTAATATCTTTTTTCCACTTTCTTTACCAATGTCTTAAAAACCAGGA
AAGTAAATGATTTCTTGTTTAAAAGTGTGATCACTCTTTTTTTGCTGGTTTGTTCA
AGTATATACAGGAAAAATGGGTGATCCGTTGATTGAAGAAATTGATGTT**CCTTCT**
TGTATACTGGATGCACCTTTACCATATTCTGTAGAGACGATGCTTGATAGAATCT
GCAAGGAGCAGGGGCAAAAACCACCGTGTACTGGCATTAGAAGGAGGCTGAGC
TCTATTGGTGAAAAAGGGTCATTAGAAATGCTCAAAATAATATCACGTCGTCCTA
TCAAGAAGAGTCTCTCTGCTTTTCTTGTTTACATGATTGATCGCTACCCGGATTGT
CTCTCCTCTTCTCTAGCCCCTTCAATTGTCTACTCAAACGCTCTTCTTCCCCTCGT
CTCTTTCCATCTCCAGGTAATTTTTCCCCTAATTTTAATTCCTTTTTCTATAATG
TTTGTGTACACAAAGTCTTTTCGCCAAAAACATGTTACATTCTAAAAATAACACAA
AACCTATTTTTTGTATGTGTGTTTAGCGTGAAAAATAAGTATATTATGTTTGGT
AGGTGAATATTTTTTCAATTCTTTTTAATGTTTGATTTATGAAAAAATAATTTTTTT
AAAAATATCTTTTATATAATTTTTGAATTGCAATTGAGAATATTTTTTAAAAATAAA
CAAAATTTTTCTTAGAAGAAAAAATAAAAAATAAGTTGATTTGGAAAATATTTT
ATCTATATAACATGGGAAAATTGAAAAATATTTTTTCAAAAAATGT

Without insertion (Susceptible type)

TTTATATAAGGAAATAATAAATTCAATATGAAAAAGATTTACATTTGACCAGAAA
TAGTTCAAATAATCAATACTTTAATTTTTTTAAGGAAAAAATAAATTTAAATAAA
GTAAAAAATCAAGACAATACCTAAAAATATATCTACATTTTTCAAATTAATAAA
TAATAATTTAAATATAAAATATATGAAAAACAATTTAAACATGTTTTTGACAAGA
TTCCCTTAAACCTATCCTCAGTGTTTCGGTCAAAAAGTATGACTTCACTTGTACGG
CATTTAACATGCAATAGTGTAGTTACTACTACCGCACTAGTTCTGTTTTCTCCCA
AAACTCATTATTTAATTTTTCTTCTTTAACAAAGTTGACGAGACAAAAGCAAAAGT
ATGAAGAATTTAATATCTTTTTTCCACTTTCTTTACCAATGTCTTAAAAACCAGGA
AAGTAAATGATTTCTTGTTTAAAAGTGTGATCACTCTTTTTTTGCTGGTTTGTTCA
AGTATATACAGGAAAAATGGGTGATCCGTTGATTGAAGAAATTGATGTTCTGGAT
GCACCTTTACCATATTCTGTAGAGACGATGCTTGATAGAATCTGCAAGGAGCAGG
GGCAAAAACCACCGTGTACTGGCATTAGAAGGAGGCTGAGCTCTATTGGTGAAA
AAGGGTCATTAGAAATGCTCAAAATAATATCACGTCGTCCTATCAAGAAGAGTC
TCTCTGCTTTTTCTTGTTTACATGATTGATCGCTACCCGGATTGTCTCTCCTCTTCT
CTAGCCCCTTCAATTGTCTACTCAAACGCTCTTCTTCCCCTCGTCTCTTTCCATCTC
CAGGTAATTTTTCCCCTAATTTTAATTCCTTTTTCTATAATGTTTGTGTACACAA
AGTCTTTTCGCCAAAAACATGTTACATTCTAAAAATAACACAAAACCTATTTTTTG

TTATGTGTGTTTAGCGTGAAAAAATAAGTATATTATGTTTGGTAGGTGAATATTTT
TTCAATTCCTTTTAATGTTTGATTTATGAAAAAATATTTTTTTAAAAATATCTTTT
ATATAATTTTTGAATTGCAATTGAGAATATTTTTTAAAAATAAACAAAATTTTTCTT
AGAAGAAAAAATAAAAAATAAGTTGATTTGGAAAAATATTTTATCTATATAAAC
ATGGGAAAATTGAAAAATATTTTTCAAAAAATGT

[*Solanum lycopersicum* chromosome ch06, complete genome

NCBI Sequence ID: HG975518.1 Range 1: 30879605 to 30880804]

Forward primer 1 (without tail): GATTGAAGAAATTGATGTTCCCTTCTGTATA
(Approximate product length with reverse primer = 101 bp)

Forward primer 2 (without tail): CGTTGATTGAAGAAATTGATGTTCTGGATG
(Approximate product length with reverse primer = 93 bp)

Common (reverse) primer: GTTTTTGCCCCTGCTCCTTG

Mi-indel: Susceptible lines have 56bp insertion as indicated by color

TATTTTACTAACGTCGTCCTACTTAATAGTTCCGTCGGGGCTTTGATGAAACAAATT
GTAGACATCTTTTTAGAACAGTAAAAGGTGTAAGAACCCTGCCAATTTGTTTGACA
CTTCTATGTATGAAGACTCTGGAGATTTGGACGTGAAGTGCACATTTCTCCGCT
CAAATTTTGCCAGAATATCCAAAATTTTAGAAACTCCAAATACTTGTTTTTATA
ATTTAACTTTGTACACTTACAAAAGAAATTCAACATTTTTTCCAAGTTATATTTAT
TCTTTCTAGGCATAGTACATTAATAATGACATTTAGCTTGACTTCAGTGGATAACT
GTGACCTTAACTTTGCT

[*Solanum lycopersicum* chromosome ch06, complete genome

NCBI Sequence ID: HG975518.1 Range 1: 2322674 to 2323023]

Forward primer 1 (without tail) (susceptible)- GGACGTGAAGTGCACATTTT
(Approximate product length with reverse primer = 70 bp)

Forward primer 2 (without tail) (resistant)- CAATTTGTTTGACACTTTCTCCGC
(Approximate product length with reverse primer = 60 bp)

Common (reverse) primer: GTTTCTAAAATTTTGGGAATATTCTGGC

Tomato SNP: Tomato Ty-3 gene alleles

Allele 1 – **C** nucleotide (resistant allele)

Allele2- **T** nucleotide (susceptible allele)

AGTAAATTCCTTTCATGGGAACAAAGTTGGAAAATGACAATCTCTTGCAATT
CATATTTGATGCTTCAGGGAGAGAGATGTGTGCCAAACTTAGTCGGCATTTTA
CTCAATGCTAAACTTTGACATTCTATGTGCATTTCACTCCCTAATTCTTAGGCT
TTTTCTCTTGAAGTCATAGACCAAATAAGGCATATCTGTCCAAGAATTTAATC
TCTCTGCTGAGCTACGGAGGAGTCCATAAAGAATACTTTATGGAACCTTTGG
GAAGTGCCTGGAAGAGACGAAACAAGTATATTTGAGGAAACGTGCAGCTC
TAAAAGGTAACAGCAGAATCAGATACTCCTATTGCATTTATATTCTTATGCTG
ATAAGTCTTGTAACATAACCATGGAATCACCCATACTGCAACCTTATATTCTT
TAGTGTCTTTTGTCTGTAATATTTAACTCCTCAGTCATAGAAAGGTAGGGAG
GAATAAGTGTCCAATTATTTCTATTACTATCTCTCATTTAATTTGTGAAACCTG
TCAGAACTTGTTTGCTGAGTCATTTCTTCAACTCTTCAAATAGTTGCTATCAA
CTATAGAGAAATGGATGATGAATGTCTAACAGCAAGGATGATATCGTCTGGG
ATACCTCTCAATGAACCTCATCTCCATG[C/T]TCGCTTGTCTAGGCTTGCAAAG
ATTGAAAGAACTAAGCTTAGAGGAGGAAAGCTTCTATAAGTGACAGTTTTT
ATCTTATGGGAACAGCTGACCCCACTGGTGTACTGGAAAGCAATGAAGTCTG
TGTTATTCTGTAAGTAGACTGAAAATCATTTTCTGTACAAGCGTTTGGAGCTT
TTATATGTTTTTTATATTCTGTGTTTCGAGAGATAAGTAACTGTTTCTTTCAATG
GCAACTGACGGATATTTATAGTTTGACCTGCTATCTGACCCCTCCTATTCT
GAGGATCTATATTTCAACAGGATGCATTGCCTTTTTGCATTCTAGATGTGTG
GCTTAACTGTTACTTGACAATAATAGTGACCCAAGGAAATCATGATGTTTAC
AAGCAAATAAATTCCTATTTACCAATGTATTTTTGTTTGTGCTTCCATCTAT
GACTTTTTTCTGTCTGCCTTCATATTTCTGCCAATGTGTAGCAACTTTAAATT
CTTTGTTTTGTAAGTTTTAACAATGATAATTGATAACAGAGGAGAGATGCTGA
AAATGACATTTACAATTATTTGAAATTATATCAAGCTAAGGTTTTCTTCCAAA
GAGAATTTAAGCTTTTAAACATGATAATTAGTT

[*Solanum lycopersicum* chromosome ch06, complete genome
Sequence ID: HG975518.1 Range 1: 30854708 to 30856007]

Forward primer 1 (without tail): CTCAATGAACCTCATCTCCAGGC

Forward primer 2 (without tail): CTCAATGAACCTCATCTCCAGGT

Common (reverse) primer: AGATAAAAAGTCACTTATAGGAAGCT

Approximate product length: 102 bp

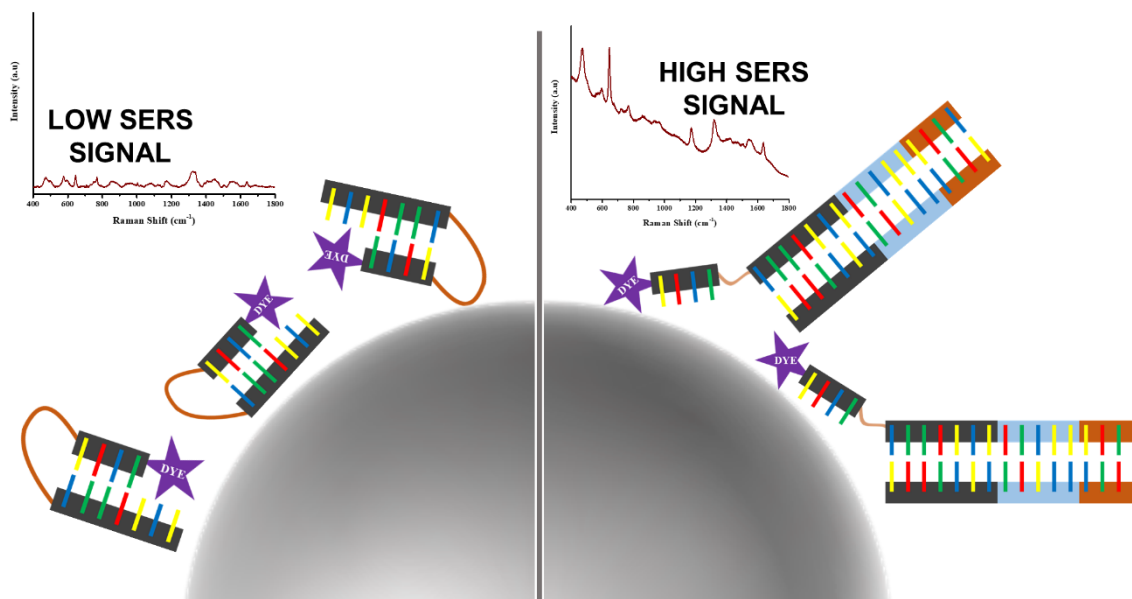


Fig. S1. Schematic representation of the functioning of a unimolecular DNA probe

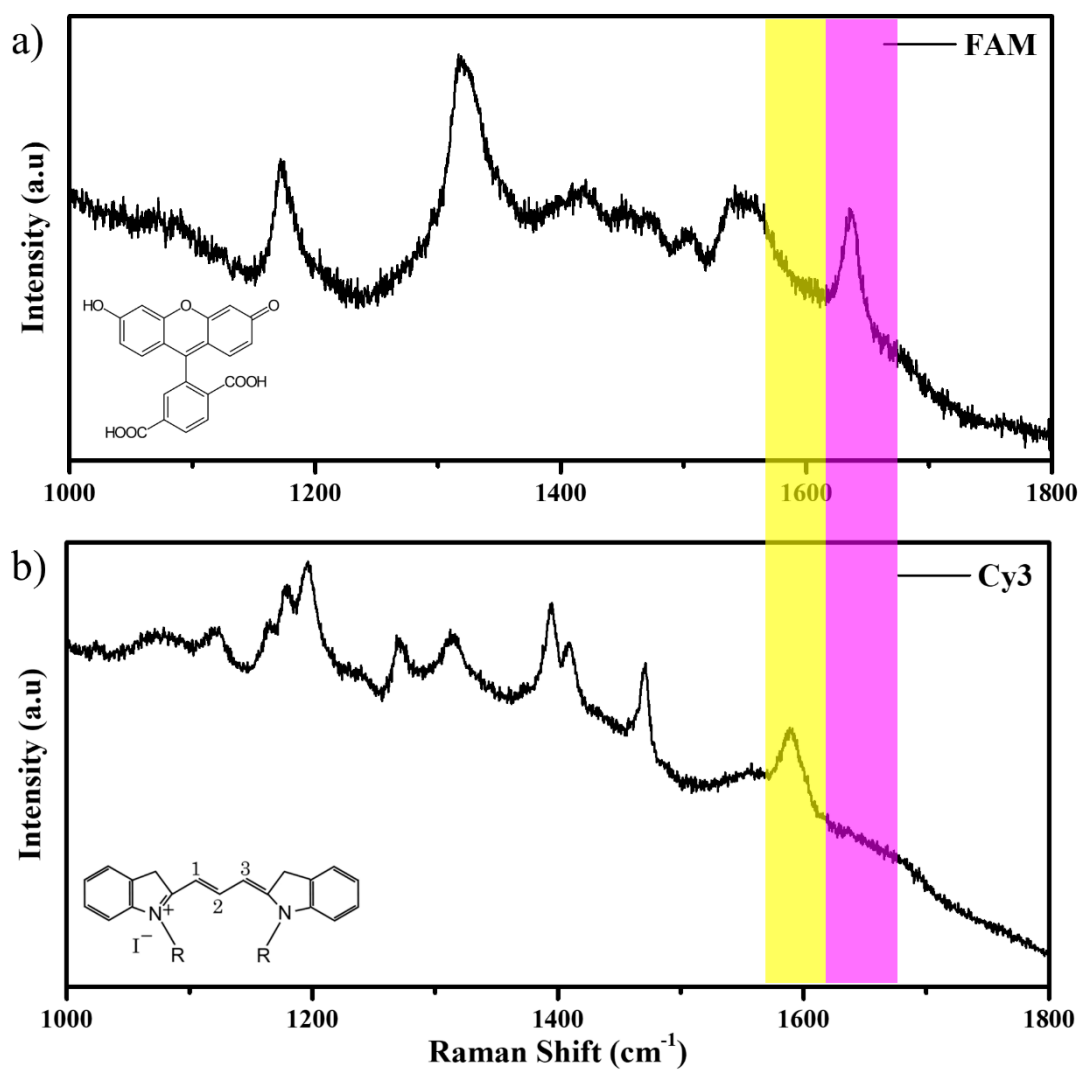


Fig. S2. Raman peaks in the SERS spectra corresponding to the a) FAM and the b) Cy3 dyes. The yellow highlighted region with a peak at 1589cm^{-1} represents the unique peak corresponding to Cy3 and the pink highlighted region with a peak at 1636cm^{-1} represents the unique peak corresponding to FAM.

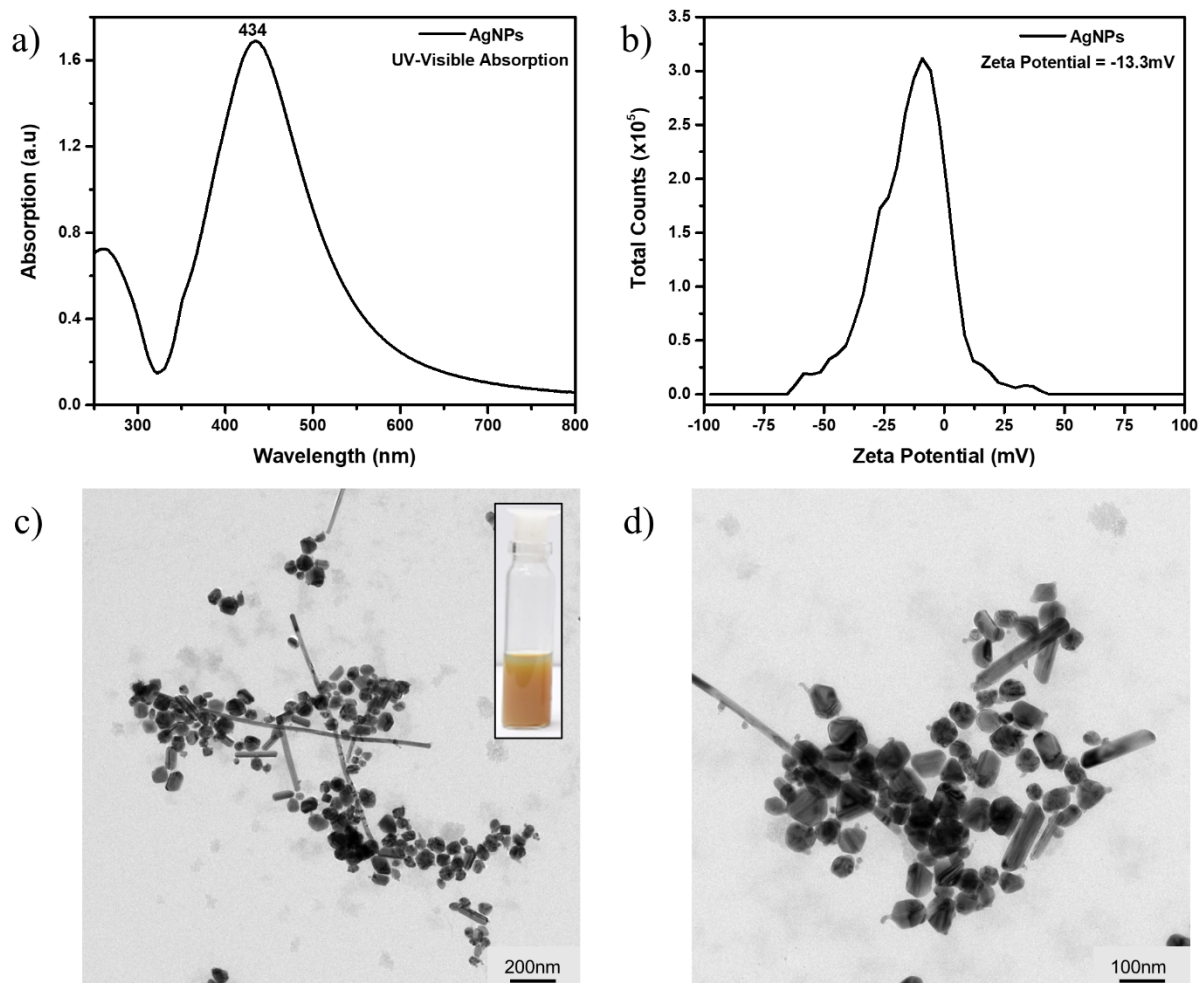


Fig. S3. a) UV-Visible absorption of the synthesized AgNPs, indicating a plasmon peak at 434 nm, b) Zeta Potential curve, with a zeta potential value of -13.3 mV, indicating negatively charge AgNPs, c) and d) TEM images of the synthesized AgNPs, Inset – the optical image of the AgNPs. The AgNPs are ranging from spherical to nano-rod-like structures.

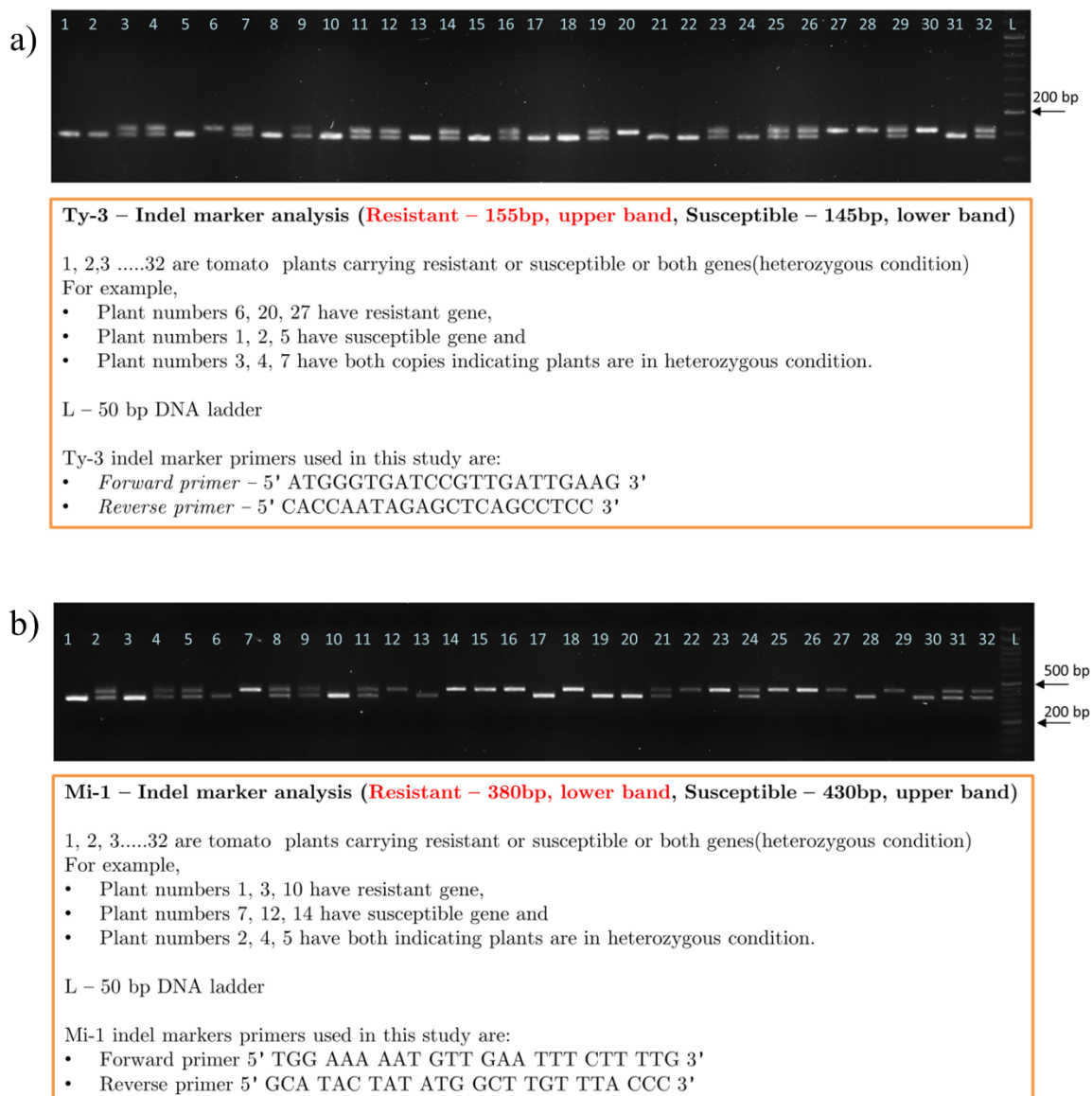


Fig. S4. Gel electrophoresis data representing the DNA of the susceptible and resistant plant varieties, in a) Ty3 and b) Mi1 gene

Table S1. Oligo sequences for Ty3 indel detection (iSp18 – hexaethylene glycol spacer)

Type	Sequence
Forward primer specific to Insertion	GTTTGATTACACCCCGTAAGATTGAAGAAATTGATGTTTCCTTCTTGTATA
Forward primer specific to Deletion	TCCGCAACAGGACAGGTGGCGTTGATTGAAGAAATTGATGTTCTGGATG
Reverse primer	GTTTTTGCCCCTGCTCCTTG
Probe for detection of the tail of insertion-specific forward primer	[6FAM]-TGCCCTTGTA-[iSp18]-TACAAGGGCACGTAGTTCAC
Probe for detection of the tail of deletion-specific forward primer	[Cy3]-GCTCACCCAC-[iSp18]-GTGGGTGAGCCTGTTACACC

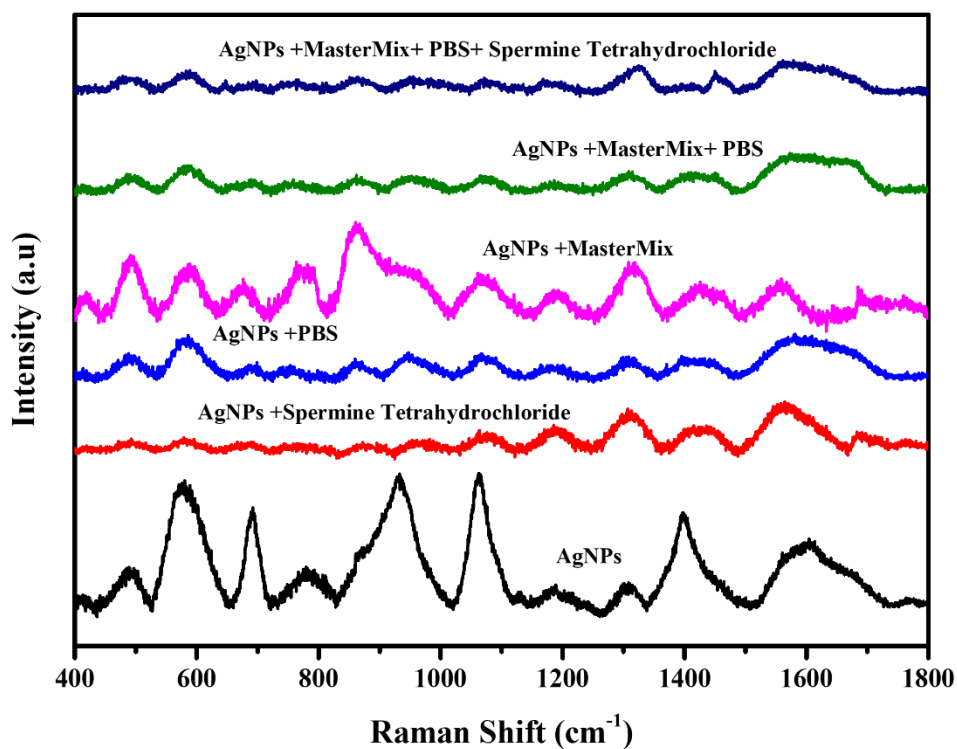


Fig. S5. Raman spectra of all the controls including AgNPs, mixtures of AgNPs with spermine tetrahydrochloride, PBS, Mastermix, Mastermix+PBS, and Mastermix+PBS+spermine tetrahydrochloride.

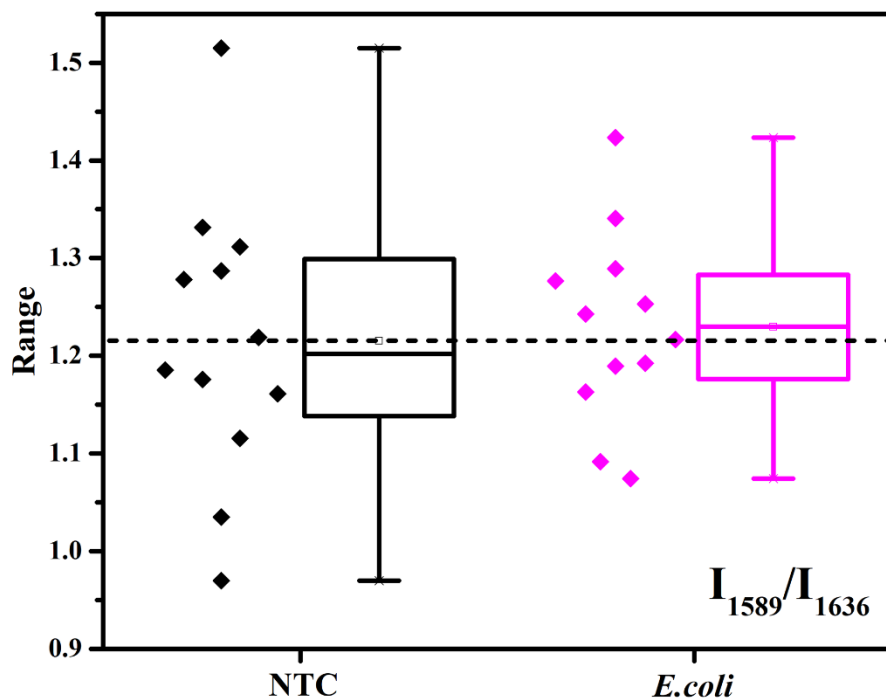


Fig. S6. Box plot corresponding to multiplexing studies of *E.coli* DNA

Table S2. Oligo sequences for Mi-1 indel detection

Type	Sequence
Forward primer specific to Insertion	CAACCTGGCGGGTATATTCAGGACGTGAACTGTCACATTTTC
Forward primer specific to Deletion	GAGGGTTAATCCCTCGGACTCAATTTGTTTGACACTTTCTCCGC
Reverse primer	GTTTCTAAAATTTTGGAAATATTCTGGC
Probe for detection of the tail of insertion-specific forward primer	[6FAM]-CGCCAGGTTG-[iSp18]-CAACCTGGCGGGTATATTCA
Probe for detection of the tail of deletion-specific forward primer	[Cy3]-ATTAACCCTC-[iSp18]-GAGGGTTAATCCCTCGGACT

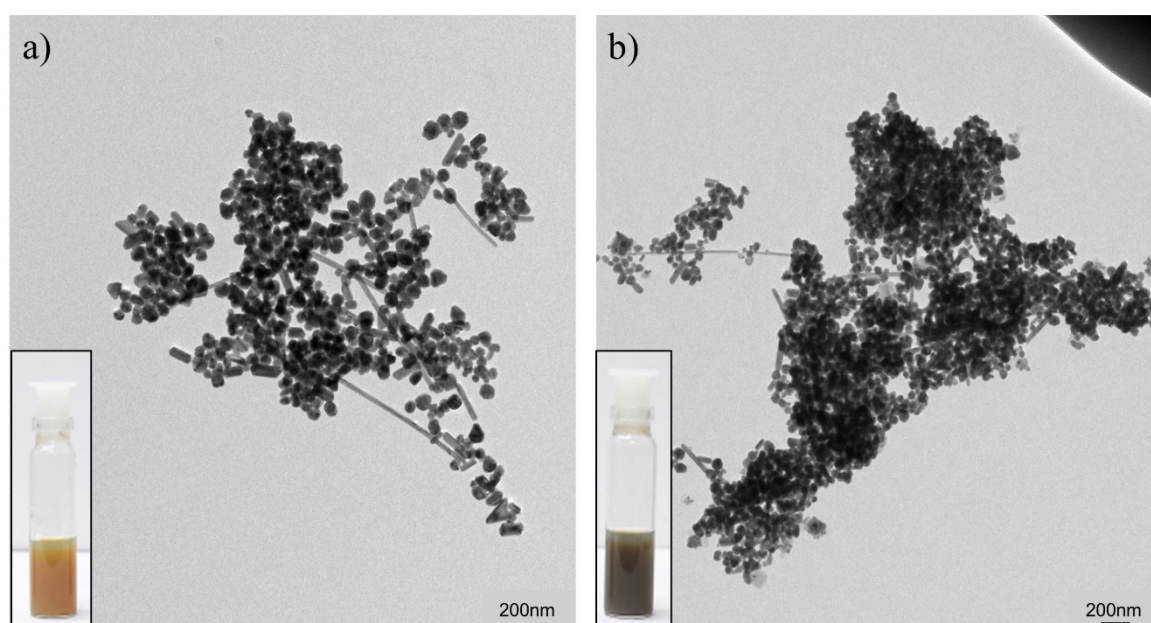


Fig. S7. TEM images of AgNPs with, a) 400 μM ST indicating aggregates in the size of 500-800 nm, and b) 0.01 M ST indicating aggregates in the size of 2-3 μm. Inset – optical image of the mixtures show, a) slight tinge of grey color of mixture, b) dark grey color of the mixture with 0.01 M ST.

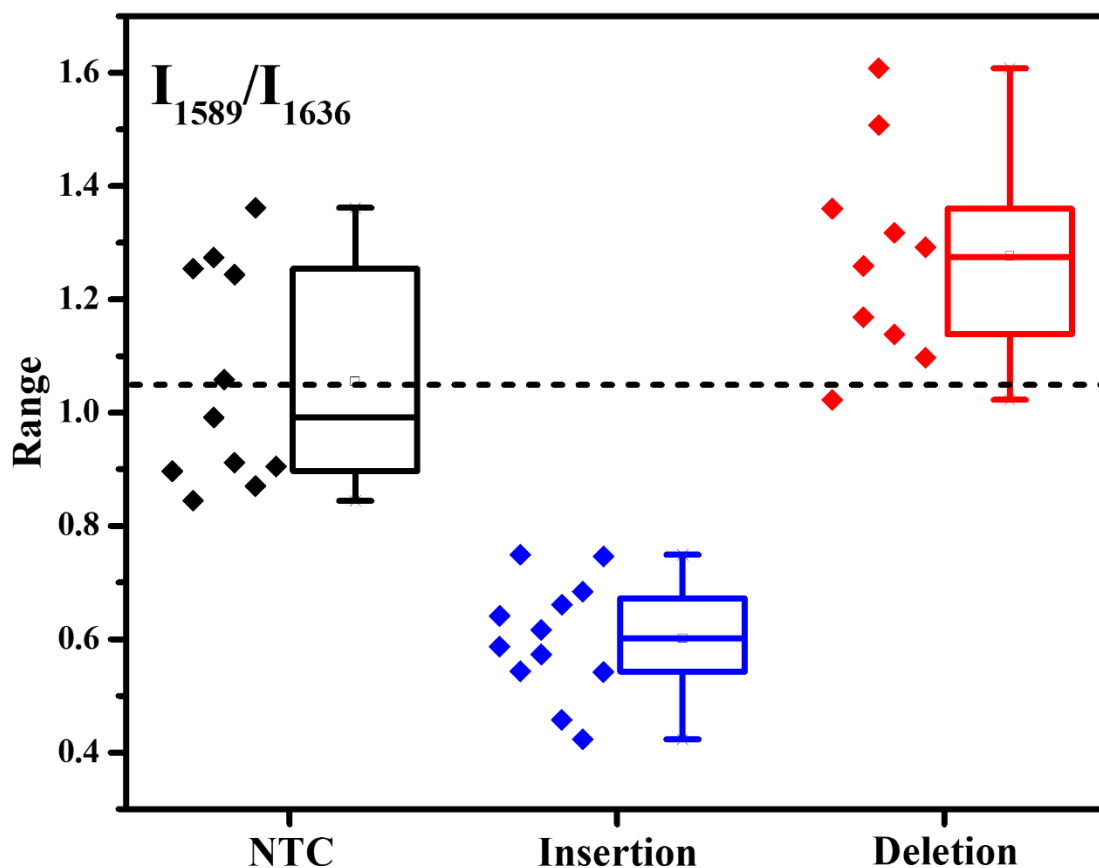


Fig. S8. Box plot for multiplexing SERS spectral analysis of the PCR products containing both FAM-tagged and Cy3-tagged probes using 0.01M ST – Intensity ratio of 1589 cm⁻¹ and 1636 cm⁻¹ peaks, blue – Resistant DNA (insertion indel), red – susceptible DNA (deletion indel)

Table S3. Oligo sequences for Ty3 SNP detection

Type	Sequence
Forward primer specific to Insertion	CAACCTGGCGGGTATATTCAGGACGTGAACTGTCACATTTTC
Forward primer specific to Deletion	GAGGGTTAATCCCTCGGACTCAATTTGTTTGACACTTTTCTCCGC
Reverse primer	GTTTCTAAAATTTTGGAAATATTCTGGC
Probe for detection of the tail of insertion-specific forward primer	[6FAM]-CGCCAGTTG-[iSp18]-CAACCTGGCGGGTATATTC
Probe for detection of the tail of deletion-specific forward primer	[Cy3]-ATTAACCCTC-[iSp18]-GAGGGTTAATCCCTCGGACT

Table S4. Comparison of existing techniques with SERS-based PCR assay

Techniques:	Gel-based methods (RAPD, RFLP)	Sequencing methods (Sanger, pyrosequencing, Mini sequencing)	TaqMan™	Fluorescent molecular beacon	PCR-based SERS detection
Features					
Detection	Endpoint	Real-time/Endpoint	Real-time	Real-time	Endpoint
Detection Platform	Fluorescence or Radioactive	Fluorescence/ Luminometric etc.	Fluorescence	Fluorescence	Raman
Specialized Equipment required	Electrophoresis chamber	Colorimeter/ Pyro sequencer/ MALDI-TOF/ Flow cytometer etc.	Fluorescence monitoring thermocycler	Fluorescence monitoring thermocycler	Normal thermocycler, Raman Spectrometer
Multiplexing Ability	Moderate	-	Moderate	Moderate	High
Cost	Low	High	Moderate	Moderate	Moderate
Specificity	Low	High	Moderate	Moderate	Moderate
Time	Time-consuming	Time-consuming	Time saving	Time saving	Time saving

References:

- Bernstein, E.; Caudy, A. A.; Hammond, S. M.; Hannon, G. J., Role for a bidentate ribonuclease in the initiation step of RNA interference. *Nature* **2001**, *409* (6818), 363-366.
- Verlaan, M. G.; Szinay, D.; Hutton, S. F.; de Jong, H.; Kormelink, R.; Visser, R. G. F.; Scott, J. W.; Bai, Y., Chromosomal rearrangements between tomato and *Solanum chilense* hamper mapping and breeding of the TYLCV resistance gene Ty-1. *The Plant Journal* **2011**, *68* (6), 1093-1103.
- Verlaan, M. G.; Hutton, S. F.; Ibrahim, R. M.; Kormelink, R.; Visser, R. G. F.; Scott, J. W.; Edwards, J. D.; Bai, Y., The Tomato Yellow Leaf Curl Virus Resistance Genes Ty-1 and Ty-3 Are Allelic and Code for DFDGD-Class RNA-Dependent RNA Polymerases. *PLOS Genetics* **2013**, *9* (3), e1003399.