SUPPORTING INFORMATION

Scanometric Detection of Tomato Leaf Curl New Delhi Viral DNA using Mono-and Bi-functional AuNPs Conjugated Oligonucleotide Probes

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EXPERIMENTAL SECTION:

Primers Design and Synthesis.

Nucleotide sequences of ToLCV New Delhi isolates were retrieved from NCBI and the sequences were aligned using ClustalW multiple sequence alignment tools using BioEdit 7.2.2 free software. From the aligned sequences, 20 and 21 nucleotide sequences were selected at highly conserved region of 5' and 3' end of CP gene sequence, respectively in order to amplify CP gene. Primer dimer analysis was performed using Oligo Analysis Tool (http://www.operon.com/tools/oligo-analysis-tool.aspx). Designed primers sequences were custom synthesized.

Similarly, the identical and its complementary sequences were selected from two different regions from the result of multiple sequence alignment of ToLCNDV sequences for the synthesis of probes and positive control. The selected sequences were subjected to self-complementation and dimer formation analyses through online tools. The designed probes (Forward probe 1 – 26 mer HS-5' -GAATT CATGTCSAAGCGWCCRGCAGA-3' and Reverse
probe 2 – 26 mer HS-5’-GGTACCATTCTTMACAGTWGCAGTGC-3’) were custom synthesized with thiol functional group at 5’ end for the preparation of conjugates.

Isolation and amplification of ToLCNDV DNA

Total genomic DNA was extracted from ToLCNDV infected tomato leaf sample based on previously described method.1 The isolated DNA was analyzed by 0.8% agarose gel electrophoresis and quantified by UV-visible spectrophotometer. 500 bp of the CP gene fragment was amplified from ToLCNDV DNA with geminivirus specific common primers using PCR. The reaction mixture was prepared to the total reaction volume of 50 µL by the addition 25 µL of 2x red dye PCR Master Mix, 22.5 µL DNase/RNase free deionized water, ToLCV specific primers (100 pM of 0.625 µL forward primer and 0.625 µL reverse primer) and 1.25 µL of template DNA. The amplification program consist the following steps which are the initial denaturation at 94 °C for 5 min followed by denaturation at 94 °C for 1 min, annealing at 60 °C for CP gene (ToLCVFP: 5’-CATGTCSAAGCGWCCRGCAGA-3’ and ToLCVRP: 5’-ATTCTTMACAGTWGCAGTGCA-3’) for 1 min, extension at 72 °C for 2 min with 25 cycles and a final extension at 72 °C for 5 min. Amplified PCR products were extracted and analyzed and quantified for further experiments.

Rolling circle amplification of ToLCNDV DNA.

The isolated DNA from samples was used as template for amplification of viral circular DNA into concatamer using TempliPhi DNA amplification kit, IllustraTM (GE Healthcare UK Limited, UK). The reaction mixture was prepared as per manufacturer’s instructions and the amplification premix (10 µL) contained 50 mM Tris-HCl (pH 8.2), 5 mM MgCl₂, 75 mM KCl, 0.1 mM dithiothreitol, 100 pM (5 µM final concentration) Thiophosphate protected random hexamer, 5 units φ29 DNA polymerase, 0.03 units pyrophosphatase and 0.1 mM dNTPs. One micro liter of DNA template was added with 5 µL of sample buffer and incubated at 95 °C for three min and cooled at 4 °C for 10 min. After incubation, 5 µL of reaction buffer and 0.2 µL of enzyme mix were added. The mixture was incubated at 30 °C for 18 hrs for amplification and incubated at 65 °C for 10 min to inactivate the enzyme. The amplified product was analyzed by 0.8 % agarose gel electrophoresis.
**Elution of Amplified DNA.** Amplified PCR and RCA products were eluted from the reaction mixture by addition of 5 μL of sodium acetate solution (pH 4.2) and 100 μL of 95% ethanol to 50 μL of PCR reaction tube. The mixture was vortexed gently and incubated at -20 ºC for 40 min. Then the mixture was centrifuged at 10,000 rpm for 20 min. The supernatant was discarded carefully and pellet was washed with 70 v/v % ethanol. After washing, the pellet was air dried at RT and suspended in 50 μL RNase/DNase free deionized water for further studies. Eluted products were electrophoresed in 1.2 w/v % agarose, visualized under the UV light and quantified using Nanodrop 2000c Spectrophotometer, Thermo scientific Inc., (USA).

**Fragmentation of PCR, RA and genomic DNA**
ToLCNDV PCR, RCA and genomic DNA were heat fragmented to reduce the size for the efficient hybridization. The complete fragmentation of dsDNA samples was standardized by heating 30 μL of dsDNA samples at 95 ºC in different time durations (0, 10, 20, 30 and 40 min) using PCR machine. Then the fragmented dsDNA samples were analyzed using 1 % agarose gel electrophoresis and the gel images were analyzed using gel analyzer software 2010 (Istvan Lazar) to determine the intensity of DNA bands before and after the heat treatment for different time durations. This strategy was used commercially for the preparation of DNA fragments from genomic DNA in the size of 500 bp – 100 bp by followings Keats and Kreatech labs procedure.²

**Synthesis and characterization of citrate capped AuNPs**

Citrate capped AuNPs were synthesized based on the previously described boiling method.³ In brief, 250 mL of round-bottom glass flask, a reflux condenser, and a large stir-bar washed with aqua regia (3: 1, Conc HCl: Conc HNO₃ v/v) followed by thoroughly washed with water. The glassware assembled on a heating mantle and a magnetic stirrer, and put the stir-bar in the flask. 100 mL of 1mM HAuCl₄ was added to flask and boiled the solution to reflux (100 ºC) with vigorous stirring. Then, 5 mL of 38.8 mM sodium citrate was added to the solution at once and continue to boiling the solution for 20 min. When, yellow color of solution turns to purple and then to deep red which indicated the formation of AuNPs. Then, the solution was cooled at RT and filter through 0.45 μm nylon filter. Finally, the solution was stored at RT in a glass container in the dark place. The solution cooled at RT and characterized by UV-visible spectrophotometry (ShimadzuUV-1600, Japan).
The size and shape of the synthesized AuNPs were analyzed by HR-TEM [(model Fei Technai G² F30 S-TWIN) with 200 kV high-resolution (UHR) pole piece]. AuNPs solution (2 µL) was dropped on a carbon coated copper grid and dried in air at RT. Then, copper grid was placed in the sample holder and HR-TEM operated at an accelerating voltage of 200 kV with high-resolution (UHR) pole piece, lattice images were recorded. Hydrodynamic diameter, zeta potential and size distribution of synthesized AuNPs were analyzed using Malvern particle size analyzer (Zetasizer Nano S). AuNPs solution (2 mL) was taken in a disposable cuvette and placed in sample holder. Particle size analyzer operated at an accelerating voltage of 100 VA at 25 °C, count rate 232 kcps with duration of 60 s. The data were recorded by Zetasizer Ver. 620.

The concentration of synthesized AuNPs was calculated theoretically with the help of particles diameter and molar concentration of gold salt solution. Assumed that NPs were spherical in shape and uniform in size, the average number of gold atoms per nanoparticle calculated using the following equation \( N = \left( \frac{R_{\text{cluster}}}{R_{\text{atom}}} \right)^3 \). Where \( R_{\text{cluster}} \) – is the diameter of NP in nanometer obtained from TEM analysis and \( R_{\text{atom}} = 0.137 \) nm – is the diameter of gold. From the result of \( N \), the amount of nanoparticles formed (\( N_{\text{NP}} \)) in a given concentration of gold salt solution (1 dm\(^3\) or 1 L of 1 mM: Moles of H\( \text{AuCl}_4 \) = \( 1 \times 1 \times 10^{-3} = 1 \times 10^{-3} \) mol) reduced by citrate was calculated through the estimation of number of gold atoms presented in a given solution using equation \( N_{\text{atom}} = \text{Moles of H\( \text{AuCl}_4 \) x } N_A \), where \( N_A - (\text{Avogadro number} - 6.022 \times 10^{23}) \). From the findings of \( N \) and \( N_{\text{atom}} \), the total number of NPs formed in given solution was calculated through equation \( N_{\text{NP}} = N_{\text{atom}} / N \). Hence, the final concentration of the gold colloid (\( C_{\text{NP}} \)) was estimated by dividing \( N_{\text{NP}} \) through Avagadro’s number (\( N_A \)): \( C_{\text{NP}} = N_{\text{NP}} / N_A \) in mol dm\(^{-3}\) (1 L).

**Preparation of Mono- and Bi-functional Signal Probes (AuNP-Probes Conjugates)**

The synthesized AuNPs solution was used for the preparation of AuNP-probe conjugates. The designed probes (Oligo probe 1 – 26 mer specific for forward strand and Oligo probe 2 – 26 mer specific for reverse strand) were custom synthesized with thiol functional group at 5’ end for the preparation of mono- and bi-functional signal probes. Complementary sequences for these two oligo probes were also designed and custom synthesized.

Signal probes 1 and 2 with mono-functional and bi-functional signal probe were prepared based Au-S chemistry. In details, 10 µL of 100 pM of forward probe and reverse probes were mixed
separately with 10 μL of 0.1 M of PBS. Then these solutions were added to 1 mL of aqueous nanoparticle solution to a final concentration of oligonucleotide probes 1 pM and incubated at RT for 24 hrs. After the incubation, the solution was subjected to “aging” process with 100 mM PBS buffer at pH 7 (containing NaCl at final concentration of 0.1 M) for an additional 24 hrs. Excess and unbound probes were removed by centrifugation at 14,000 rpm for 30 min. While, the supernatant was discarded, the red oily precipitate was washed twice with 10 mM phosphate buffer (pH 7) containing 0.3 M NaCl solution (0.3 PBS) by successive centrifugation and redispersion and finally they were redispersed in fresh 500 μL of 0.3 M PBS. Similarly, bi-functional signal probe (AuNP-probe 1&2) were prepared by equally mixing of 5 μL of 100 pM of oligonucleotide probe 1 and 5 μL of 100 pM of oligo probe 1 with 10 μL of 0.1M of PBS and the same was added to 1 mL of aqueous nanoparticle solution. The synthesized probes were characterized by UV-visible spectrophotometer, HR-TEM, DLS, zeta potential analysis and Raman 11 (Confocal Raman Microscopy).

**UV-Visible Spectrophotometer Analysis**

The optical absorbance property of the prepared mono-functional signal probes 1 and 2 (AuNP-Probe conjugates 1 and 2) and bi-functional signal probe (AuNP- equal mixture of oligo probe 1&2) was analyzed using Shimadzu UV-1600 spectrophotometer. For this, 300 μL of AuNPs was taken as a reference and 0.3 M PBS with AuNPs as a control. Prepared signal probes were taken in 350 μL capacity of 1 cm light path quartz cuvette and kept at the sample holder with distilled water as a blank. The optical absorbance spectrum was recorded from 200 nm to 1000 nm.

**HR-TEM Analysis**

The size and shape of prepared signal probes were analyzed by HR-TEM (Fei Technai G2 F30 S-TWIN). 2 μL of signal probes (mono and bi-functional) solution were dropped onto a separated carbon coated copper grid and dried in air at RT. Then, copper grid was placed in the sample holder and the HR-TEM was operated at an accelerating voltage of 200 kV with high-resolution (UHR) pole piece. Lattice images were recorded.

**DLS Analysis**

Hydrodynamic diameter, zeta potential and size distribution of prepared signal probes (mono and bi-functional) were analyzed using Malvern particle size analyzer (Zetasizer Nano S, UK). Two mL of conjugates solutions were taken in a separated disposable cuvette and placed in sample
holder. Particle size analyzer was operated at an accelerating voltage 100 VA at 25 °C and count rate 232 kcps with duration of 60s. The data was recorded by Zetasizer Ver. 620 software.

**Agarose Gel Analysis**
Prepared AuNP-signal probes were characterized using agarose gel electrophoresis to understand stability and conjugation efficiency. In detail, 20 μL of AuNP-signal probe conjugates were mixed with 6 % sucrose and loaded in to 1.2% agarose gel allowed to run the electrophoresis at 100 mV for 40 min with 0.5X TBE running buffer. After the completion of electrophoresis, gel image was captured using a digital camera. Intensity of bands were analyzed using ImageJ software.⁷

**Calculation of Immobilized Oligonucleotide Probes on AuNPs Surface**
Prepared AuNPs- oligo probe conjugates were treated with β– mercaptoethanol (final concentration of β– mercaptoethanol 12 mM was prepared using 0.1 M NaCl) and kept overnight.⁸ After incubation, the solutions were centrifuged at 14000 rpm for 30 min. Optical absorbance of the supernatants was measured at 260 nm using UV-visible spectrophotometer (UV-1600, Shimadzu). The concentrations of probes in supernatants were calculated based on the absorbance with following standard value of ssDNA oligomers.

\[ C = \frac{A}{(e \times l)} \]

where:
- C: the concentration of the nucleic acid, in μg/mL,
- A: Absorbance (OD260).
- l: width of the cuvette which used to hold the solution, in cm, usually is 1cm
- e: Extinction coefficient of oligoes [1 OD260Unit = 20 μg/ml for single stranded oligos (ssOligo)]

With the help of Mw and molar concentration of the probe, we calculate the numbers of probe in the given volume of solution (Molar concentration and Mw of probe are obtained from synthesis report).

Moles of probe (Mprobe) = Concentration (in M) x Volume (in L)
Number of probes Nprobe = Mprobe x NA
Number of probe attached on per AuNP = Nprobe/ NNP
RESULTS:

Table S1. ToLCNDV specific signal probes, capture probes and its complementary sequences

| Name                        | Sequences                                                                 |
|-----------------------------|---------------------------------------------------------------------------|
| Signal probe 1 (26 nt)      | 5’-SH-GAATTCATGTC\text{\textsc{s}}AAGCGWCCR\text{\textsc{r}}GCAGA-3’       |
| Signal probe 2 (26 nt)      | 5’-SH-GGTACCATTCTTMACAGTWGCAGTGC-3’                                      |
| Capture probe 1 (27 nt)     | 5’-CGTGCTGCTG\text{\textsc{y}}CC\text{\textsc{c}}CATTGTCCCGGYC-PO\text{\textsc{4}}-3’ |
| Capture probe 2 (27 nt)     | 5’-CATAACACTRTTMGTRTGATTCTTRGC-PO\text{\textsc{4}}-3’                   |

Positive Controls for DDH Assay

|                     | Sequences                                                                 |
|---------------------|---------------------------------------------------------------------------|
| C-ssDNA 1 (38 nt)   | 5’PO\text{\textsc{4}}-GAAATGATTATATATCTGCGG\text{\textsc{y}}GG\text{\textsc{w}}C\text{\textsc{t}}TS\text{\textsc{g}}GACATG\text{\textsc{a}}ATT\text{\textsc{c}}-3’ |
| C-ssDNA 2 (38 nt)   | 5’PO\text{\textsc{4}}-GATAATGAGCCCAGC\text{\textsc{a}}CTGC\text{\textsc{w}}ACTG\text{\textsc{k}}KAAGAATGG\text{\textsc{t}}AC-3’ |

Positive Controls for SDH Assay

|                     | Sequences                                                                 |
|---------------------|---------------------------------------------------------------------------|
| C2-ssDNA 1 (73 mer) | 5’-TCTGCGG\text{\textsc{w}}CG\text{\textsc{c}}TT\text{\textsc{g}}GACATG\text{\textsc{a}}ATT\text{\textsc{c}}-T (20 nt)- |
|                     | GRCGC\text{\textsc{g}}GAC\text{\textsc{t}}G\text{\textsc{c}}GAC\text{\textsc{g}}GACACG-3’ |
| C2-ssDNA 2 (73 mer) | 5’-G\text{\textsc{c}}ACTG\text{\textsc{c}}WACTG\text{\textsc{k}}AAGAATGG\text{\textsc{t}}AC- T (20 nt)- |
|                     | GCYAA\text{\textsc{a}}GAAT\text{\textsc{c}}\text{\textsc{y}}ACK\text{\textsc{a}}AYAGT\text{\textsc{g}}TTATG-3’ |

**PCR based amplification of ToLCV DNA fragments**

Symptomatic tomato leaf samples collected from the field were brought to laboratory for the diagnosis of viral infection. Total genomic DNA was isolated from all the samples and the presence of genomic DNA was confirmed by 0.8 % agarose gel electrophoresis. Figure S1, agarose gel image shows the bright DNA bands in all the lanes under UV transilluminator to EtBr staining. Lane 1 shows 1 kb DNA marker, lane 2 and 3 for Dharmapuri, lane 4 and 5 for Thiruvannamalai, lane 6 and 7 for Kanchipuram, lane 8 and 9 for Tiruvallur and lane 10 and 11 for Perambalur districts samples DNA, respectively. The DNA quantified and purity was checked using UV- visible spectrophotometer by measuring the absorbance at 260 nm and the ratio of absorbance at 260 nm/ 280 nm respectively. The amount of DNA was found to be ~ 50 μg per two leaf disc leaf sample (~50 mg). Isolated total genomic DNA containing both plant and viral genome was used as a template for PCR and RCA reaction studies.9,10
Figure S1. Agrose gel (0.8%) analysis of isolated total genomic DNA from collected tomato leaf samples. Lane M - 1 kb DNA ladder; Lane 1&2 - Dharmapuri, Lane 3&4 - Thiruvannamalai, Lane 5&6 - Tiruvallur, Lane 7&8 - Kanchipuram and Lane 9&10 - Perambalur districts samples DNA.

ToLCV infection in the Tomato leaf samples were identified by PCR method using ToLCV CP gene specific primers (ToLCVFP/ToLCVRP). In order to overcome the nucleotide diversity among the specific gene sequence of ToLCV of the same isolates, degenerate primers were designed. A set of forward and reverse primers were custom synthesized to amplify 0.5 kb fragment of CP of ToLCV – A component. The presence of 0.5 kb PCR amplified DNA fragments from CP gene was analyzed using 1.2% agarose gel electrophoresis (Figure S2). The amplified PCR products in all the lanes (2-6) were found to be exactly equal to 500 bp DNA ladder band of lane 1 (100 bp ladder). Amplification of DNA fragments occur only in the presence of complementary sequence for primers in the sample template DNA. In the absence of complementary sequence of primers in sample template, DNA primers annealing and amplification will not take place. This could be due to the primer annealing by exact base pairing with its complementary sequence in template DNA. PCR results in this study confirm that the isolated DNA from collected samples had viral DNA which is due the ToLCV infection in tomato plant samples collected from five districts in the state of Tamil Nadu. Among this five,
one sample (Thiruvannamalai) PCR products were taken the further sequence analysis for the identification of virus.

**Figure S2.** Agarose gel (1.2 %) image lane 1– 100 bp DNA ladder, lane 2- 6 were PCR amplified ToLCNDV CP gene DNA fragments of from Dharmapuri, Thiruvannamalai, Kanchipuram, Tiruvallur and Perambalur districts tomato samples respectively. The amplified PCR products in all the lanes were found to be exactly equal to 500 bp DNA ladder band of lane 1 (A).

RCA is used to amplify the full genome of circular DNA (plasmid, ds and ss circular DNA and RNA genomes of virus) with the help of φ29 DNA polymerase in in-vitro.\(^\text{11}\) Full genome of ToLCNDV was amplified in the form of concatamers from the total DNA isolated from Thiruvannamalai sample through RCA and the products were analyzed by agarose gel electrophoresis (Figure S3). Agarose gel image shows the presence of RCA product in lane 1 and 2 in which, the product was found to be more than 10 kb in size when compared with 1 kb DNA marker in lane M (GeneRuler 250 bp to 10,000 bp from Thermo Scientific, India) which were located near by the loading well of gel. RCA product is formed only in the presence of circular DNA template in the sample DNA, because circular DNA can only act as a template for RCA. The high molecular weight of dsDNA concatamers is obtained due to the tandem- repeat
amplification of 2.7 kb size viral genome by the action of φ29 polymerase through the multimeric geometry replication (multiple replication site on a single template).\textsuperscript{12,13}

Figure S3. Agarose gel (1 \%) analysis of RCA product of ToLCNDV from Thiruvannamalai sample. Lane M – 1 kb DNA ladder and Lane 1& 2 - RCA product with the size is more than 10 kb.
Figure S4. Synthesis of citrate capped AuNPs through boiling method. (a) Intense red color solution of AuNPs and corresponding UV-visible spectra shows maximum absorption at 520 nm (b). HR-TEM image shown dispersed spherical shape ~19 nm diameter of AuNPs, (c) and its average hydrodynamic diameter 30 nm in DLS analysis (d) (This figure is already published and permission obtained from RSC Advance).⁴
Figure S5. Agarose gel electrophoresis analysis of AuNP conjugated mono-and bi-functional signal probes. (a) Red color bands were observed in lane 2, 3 and 4 were represent the ssoligo probe functionalized AuNPs (spherical nucleic acid) and AuNPs shearing was found lane 1 indicates un-modified AuNPs. (b) Band intensities signal probes 1, signal probe 2, bi-functional signal probe and AuNPs.
Figure S6. Quantification of immobilized ssDNA on amine modified GS surface. (a) Standard bar graph of optical absorbance at 260 nm by different concentrations of ssDNA (0 M, 1 fM, 100 fM, 1 pM, 100 pM and 1 nM) and corresponding optical absorbance spectra given as inset image. (b) Bar graph of estimated concentration of complementary ssDNA 1, 2 and 1&2 immobilized on GS surface from 1 nM concentration.

PCR amplified, RCA ToLCNDV DNA and isolated total genomic DNA from infection suspected plant were also immobilized on the amine functionalized GS surface to perform the detection of specific nucleotide sequence through scanometric based nanoassay method. Prior to immobilization of DNA samples, it should be fragmented into small size to increase the number of 5′ terminal phosphate groups and increase the efficiency of immobilization as the number of functional phosphate groups at 5′ end of DNA and length of the DNA are the important factors determining the efficiency of DNA immobilization on the surface.14 Therefore, PCR, RCA and genomic DNA were fragmented by heat treatment.15 Heat fragmentation profiles of PCR, RCA and genomic DNA samples at 95 °C for different time durations (10, 20, 30 and 40 min) are shown in Figure S7 a-c, respectively. Intensity of PCR DNA band at 0 min was found to be 172.8 (a. u.) after 10 min of heat treatment, the intensity of band was decreased to 113.3 (a. u.). While increasing the time duration of heat treatment from 10 to 20, 30 and 40 min, the intensity of band was gradually decreased to 111.9, 110.5 and 95.0 (a. u.), respectively (Figure S7 a). Fragmentation of RCA DNA at different time interval is presented in Figure S7 b. At 0 min the band intensity was found to be 181.6 (a. u.) which was gradually decreased to 160.03, 157.2,
140.2, and 84.34 (a. u.) after 10, 20, 30, and 40 min heat treatment, respectively. The genomic DNA fragmentation at different time periods and band intensity (Figure S7 c) at 0 min was found to be 155.8 (a. u.). After the heat treatment of genomic DNA for 10, 20, 30 and 40 min, the band intensity was decreased considerably to 128.9, 124.7, 120.4 and 74 (a.u.), respectively. The complete disappearance of band intensity in 40 min clearly indicates the complete fragmentation of DNA in a wide range of size. Heat fragmentation efficiency was found to be higher in genomic DNA than RCA and PCR products reflected by the band intensity. Intensity of genomic DNA was found to be lower than the RCA and PCR products after 40 min heat treatment (Figure S7 d). The corresponding agarose gel images also confirm the disintegration (Figure S8) and indicate that the larger size DNA (RCA and genomic DNA) gets fragmented completely than the small one (PCR) DNA. The final size of fragmented DNA was found to be from 100 bp – few kb in size. The breakdown of DNA occurred due to heat and pressure generated by heating of DNA suspended medium at high temperature. This thermal exposure can break covalent bond within each DNA strand.16
Figure S7. Analysis of fragmented DNA samples for different times of incubation (0, 10, 20, 30 and 40 min) at 95 °C. Fragmentation of PCR amplified DNA (A), RCA DNA (B) and genomic DNA (C).

Figure S7 (Cont.). (d) Graphical representation of band intensity of PCR DNA (black line with square symbol), RCA DNA (red line with circle symbol) and Genomic DNA (blue line with triangle symbol) after heat based fragmentation in different times.
Figure S8. Agarose gel analysis of completely fragmented PCR, RCA and genomic DNA after 40 min incubation at 95 °C. Lane 1, 2 and 3 unfragmented PCR, RCA and genomic DNA as control, respectively. The size of genomic and RCA DNA was found to be more than 10 kb in size and PCR DNA was in 0.5 kb in size. Lane 4 – 100 bp marker DNA. Lane 5, 6 and 7 are completely fragmented PCR, RCA and genomic DNA respectively. These lanes show the total sheared DNA without any sharp band which indicated that the DNA was fragmented completely in a broad range of size.

The completely fragmented DNA was efficiently immobilized onto the amine functionalized GS surface due to the presence of increased number of 5′ free phosphate group than the untreated DNA samples. As discussed already EDC based immobilization method was used to immobilize the fragmented dsDNA ToLCNDV sample. Any one strand of 5′ phosphate group of dsDNA was immobilized onto the surface at a time and the opposite strand was left as such in due anti-parallel orientation of dsDNA and this will not to be immobilized. This immobilized dsDNA fragment was converted to ssDNA through alkaline treatment using NaOH. Where dsDNA fragments were denatured into ssDNA, un-immobilized strand was removed by frequent washing using the washing solution. The amount of RCA and genomic DNA immobilized onto the
surface was 1.98 μg and 2.43 μg respectively from 2.01 μg RCA DNA and 4.14 μg genomic DNA (Figure S9). However, the size of fragments was not uniform in genomic and RCA samples that lead to differences in the immobilization efficiency. While the DNA length is one of the important parameters determining the degree of DNA immobilization on the solid surface, larger size DNA fragments have high tendency to form a self coiled secondary structure than the small DNA fragments and it could occupy more space than the smaller ones and may undergo nonspecific interactions with the solid surface. All these interfere in the immobilization of DNA leading to decrease in the degree of immobilization. Therefore, fragmentation is necessary for efficient immobilization of larger size DNA (both genomic and RCA DNA).

Figure S9. Estimated amount of PCR, RCA and Genomic DNA were immobilized on GS surface. Black bar represents concentration of DNA taken for the immobilization and red bar indicates the amount of DNA immobilized on the amine modified surface.

Topology of ssDNA immobilized GS surface was analyzed by AFM (Figure S10). The AFM image of ssDNA immobilized GS surface had a surface roughness of RMS 33.8 nm which is 16 times greater than the surface roughness of unmodified GS and 5 times higher than the amine modified GS. The increased roughness of GS surface is mainly due to the ssDNA immobilization and ssDNA appearing in a bulb like shape on the surface. This is due to the highly flexible nature of ssDNA leading to the formation of self folding or compressed bulb like structure through
physical or weak interactions. Huang et al. \textsuperscript{17} observed a bulb like structure of immobilized calf thymus DNA on amine modified mica surface and concluded that the long DNA strand undergo self folding by electrostatic interaction to form bulb like structure.

**Figure S10.** AFM characterization of DNA immobilized GS surface. Increased surface roughness and bulb like structures indicates the immobilized ssDNA on GS.

**Figure S11.** Specificity analysis of mono-functional signal probe 1, 2 and bi-functional signal probe with target and non-target DNA through DDH and SDH based scanometric methods.
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