**Supplementary Information for**

**Rapid differentiation of SARS-CoV-2 variants on a nanotechnology enhanced DNA-chip**

Ying Liu\*, Yang Yang\*, Guanghui Wang\*, Ruibin Hu, Pan-Lin Shao, Jiahu Tang, Ziyi Xu, Yan Liu, Hongjun Xiao, Jiahui Lv, Jingkai Yang, Dan Niu, Meijie Tang†, Jing Yuan†, Hongjie Dai† and Bo Zhang†

\* The authors contribution equally

Correspondence to: zhangb3@sustech.edu.cn; hdai@stanford.edu; yuanj2020@mail.sustech.edu.cn; meijie.tang@nirmidas.com

**This PDF file includes:**

Supplementary Text

Supplementary Tables 1 to 5

References *38*-46

**Other Supplementary Materials for this manuscript include the following:**

Extended Data Fig. 1 to 9

Extended Data Table 1

Supplementary Text

**Supplementary Discussion 1. Surface chemistry modification on pGOLD substrate**

The gold nanostructure of pGOLD substrate was imaged by Scanning electron microscopy (SEM) (Extended Data Fig. 7e). The distance between the detection fluorophore and plasmonic gold film would affect the fluorescence enhancement and the signal-to-noise ratio to a certain extent. We first performed surface amination on the pGOLD substrate by terminal thiol and amine group modified linker (HS-linker-NH2), then converted the amine groups to maleimide groups by sulfosuccinimidyl-4-(*N*-maleimidomethyl) cyclohexane-1-carboxylate (Sulfo-SMCC) (Extended Data Fig. 1a)20,38. The 5’-thiol and 3’-Cy5 modified DNA (2.5 μM) was used to measure the fluorescence intensity on glass, pGOLD substrate and cysteamine, poly-ethylene glycol 1,000 (PEG 1,000, Mw = 1,000), PEG 5,000 (Mw = 5,000), bovine serum albumin (BSA) modified pGOLD substrate (Extended Data Fig. 1c).

For FEMMAN assay, the 5’-thiol modified DNA probe was immobilized on these six substrates followed with maleimide group blocking with 6-mercaptohexan-1-ol (MCH), the complementary DNA target with 5’-biotin was captured by DNA probe with following IRDye800 labeled streptavidin identification (Extended Data Fig. 1b). Four-layer chemical modification, HS-linker-NH2, Sulfo-SMCC, double strand DNA and IRDye800 labeled streptavidin was constructed between the fluorophore and the pGOLD substrate. The distance between the fluorophore and pGOLD substrate was mainly regulated by HS-linker-NH2 (cysteamine, PEG 1,000, PEG 5,000 and BSA). We observed that when 5'-thiol modified DNA was directly printed on the pGOLD substrate, the condensed thiol groups would etch the plasmonic gold film away from the glass substrate (Extended Data Fig. 1c, pGOLD substrate). Surface chemical modification with HS-linker-NH2 would protect the plasmonic gold nanostructure, and as the molecular weight of HS-linker-NH2 augmented, the fluorescence signal gradually increased (Extended Data Fig. 1c, d). In addition, the surface chemical modifications had influence on the signal-to-noise ratio. We observed different degrees of fluorescence signal spillover around the printed DNA spots, it would be potentially due to the different loading capacity of the HS-linker-NH2 molecules (Extended Data Fig. 1c). Comprehensively considering the fluorescence enhancement and the signal-to-noise ratio, we finally chose BSA as the HS-linker-NH2 molecule for surface chemical modification on pGOLD substrate.

**Supplementary Discussion 2. Binding buffer optimization**

The ion concentration in binding buffer is a significant factor in DNA duplex formation. We first performed the hybridization in Tris-EDTA buffer (TE) and obtained insufficient fluorescence signal. Then we combined some salts and buffers commonly used in DNA hybridization to screen a suitable binding buffer for FEMMAN assay, including TE buffer containing 0.1 M NaCl, 0.5 M NaCl, 1 M NaCl, 0.1 M MgCl2, 0.5 M MgCl2, 1 M NaCl and 0.1 M MgCl2, 0.5 M KH2PO4, 0.5 M K2HPO4, 2 × citrate buffered saline (SSC buffer), 5 × SSC buffer, 6 × SSC buffer, 6 × SSC buffer containing 0.1 M NaCl, 1 M NaCl, 0.1 M MgCl2, 0.5 M KH2PO4 and 0.5 M K2HPO4 (Extended Data Fig. 1e)39,40. Furthermore, we verified the effect of concentration of DNA probe on the fluorescence signal, and observed the decrease of fluorescence signal as the DNA probe concentration increased when the probe concentration was > 2 μM. It’s potentially due to the steric hindrance caused by dense DNA probes and aggregation-caused quenching of fluorophore (Extended Data Fig. 1e)41. The optimized condition was TE buffer containing 1 M NaCl as binding buffer and spotting DNA probe at 2 μM.

**Supplementary Discussion 3. DNA hybridization assay on FEMMAN chip**

A BSA layer with maleimide functional group was constructed onto the surface of the pGOLD (Extended Data Fig. 1a). A small volume of 2 μM thiolated DNA probe solution was distributed onto the maleimide group activated pGOLD substrate as microarray spot. A spacer containing 10 Adenosine bases (dA10) was inserted between DNA probe and thiol group to enhance hybridization efficiency (Extended Data Fig. 8a). Compared with a longer spacer with 30 Adenosine bases (dA30), the fluorescence signal with dA10 spacer is stronger (Extended Data Fig. 8). For single DNA target detection, triplicate DNA probe spots were microarray printed as an array for profiling one sample. After microarray printing, free maleimide groups were quenched by 0.01% mercapto-hexanol, following with incubation of biotinylated target DNA and IRDye800 labeled streptavidin (Extended Data Fig. 1b).

Serial dilutions of DNA target from 2 nM to 2 fM plus blank control were applied to a set of DNA microarrays. DNA microarrays on glass slides and evaporated gold film were also constructed in the same way and applied for DNA detection for comparison. After labeling with IRDye800, DNA microarrays on these substrates were scanned for IRDye800 fluorescence image (Extended Data Fig. 2a). The amount of hybridized DNA target was proportional to the abundance of DNA target in solution and was reflected by IRDye800 fluorescence intensity (Extended Data Fig. 2a, b). Compared to traditional DNA microarray on glass substrate, near-infrared fluorescence enhancement of IRDye800 afforded by plasmonic gold film boosted IRDye800 fluorescence signal to > 1,000-fold, expanded dynamic range of DNA microarray by 2-3 orders of magnitude and pushed DNA detection to < 20 femtomolar range (Extended Data Fig. 2b). Unlike fluorescence enhancement on our plasmonic gold film, evaporated gold film with continuous gold structure quenched IRDye800 fluorescence, resulting in poor sensitivity for DNA microarray (Extended Data Fig. 2b).

**Supplementary Discussion 4. Simplified alternative route by asymmetric RPA**

Asymmetric RPA is a type of amplification that involves different ratios of primer-F / primer-R. Firstly, the dsDNA product is obtained with the primer pair. As the reaction continues, one primer in relatively lower amount is exhausted, and single-stranded DNA (ssDNA) is produced by the other primer using the freshly synthesized dsDNA as template42. We performed a one-step asymmetric RPA for SARS-CoV-2 viral RNA amplification with the ratio of 5’-biotin labeled forward primer / 5’-phosphate-reverse primer at 20: 1, and loaded the amplified samples onto FEMMAN chip directly without exonuclease digestion (Extended Data Fig. 3a). The sensitivity of FEMMAN assay combined with asymmetric RPA was about 50 copies per reaction, which was inferior to FEMMAN-RPA assay combined with Lambda exonuclease (Extended Data Fig. 3b, c). It’s mainly because the amplified ssDNA product of asymmetric RPA was insufficient due to the linear increase of amplicon, while the amplicon of RPA with equal amount of forward primer and reverse primer increased exponentially43. While for some conditions where single copy sensitivity is not required, the asymmetric RPA would be a simplified alternative route.

**Supplementary Discussion 5. Primer optimization in RPA system**

The sequence of probes was based on the mutation site and corresponding primers were designed according to the upstream and downstream conserved regions of the target (Fig. 2b). The forward primer is 4 bases away from the probe, while the reverse primer has an overlap of 4 bases with the probe. This is mainly because the overlap between the reverse primer and the probe will not introduce non-specific signals, while the forward primer with biotin tag should not be cross-linked with the probe to avoid non-specific signals from excess forward primer. We observed an inverse proportion relationship between the sensitivity of FEMMAN-RPA and the length of the amplicon, especially the part of amplicon between the target site and the 3’ end, which means the reverse primer should be close to the target site but with no influence on target identification. Here we used the optimized reverse primer location with 4 bases overlap of the probe (Extended Data Fig. 8). The possible explanation is the steric hindrance caused by the 3’ end of the amplicon as the rigid structure formed by double-stranded DNA (the blue part in Fig. 2a), which would reduce the amount of amplicon bound to the probe, leading to insufficient signals.

To improve the sensitivity of FEMMAN-RPA assay, we explored the influence of the amplicon structure. We designed two pairs of forward primer (FP) and reverse primer (PR), and grouped them to form 4 types of amplicons (Type 5, 6, 7 and 8, sequences and target site in Table S4, amplicon structure in Extended Data Fig. 8c)44,45. The FP1 & RP1 were away from the probe binding site and FP2 & RP2 were close to the probe binding site. We performed the RPA amplification with the 4 types of primer pairs and verified the performance in FEMMAN assay, the IRDye800 fluorescence image of the 4 types of amplicons showed that the fluorescence intensity of amplicon formed by FP2 & RP2 primer pairs was significantly higher than the others (Extended Data Fig. 8c, d). The FP2 was 4 bases away from the probe binding site, while the RP2 had an overlap of 4 bases with the probe binding site. We observed an inverse proportion relationship between the fluorescence intensity and the length of the amplicon, especially the length of reverse primer (Extended Data Fig. 8c, d). The possible explanation was the steric hindrance caused by the 3’ end of amplicon.

**Supplementary Discussion 6. Specificity of FEMMAN assay without amplification**

The specificity of FEMMAN was demonstrated through probing 15-plexed DNA microarray (Extended Data Fig. 4a) with a selected mixture of DNA target (labeled as target A, B, C, D) with length varied from 20 nt to 23 nt (Supplementary Table 4). Single and double nucleotide mismatch version of Probe C were included in the 15-plexed microarray to examine DNA hybridization selectivity for close DNA sequences (Extended Data Fig. 4a). DNA target A only; a mixture of target A and B; target A, B and C; target A, B, C and D were applied to the multiplexed DNA microarray separately at 200 fM or 20 fM for each target without amplification, a concentration well below the detection limit of DNA microarray on traditional glass substrate. Sensitivity of DNA detection in the multiplexed array was the same as single DNA detection, with fluorescence signal on non-specific DNA probe at background level (Extended Data Fig. 4b). When target C was introduced for DNA hybridization, fluorescence intensities on single and double nucleotide mismatch version of Probe C were ~ 10% and 5% of intensity on probe C, demonstrating the capability of FEMMAN chip in discriminating DNA hybridization to complementary sequence versus mismatch versions (Extended Data Fig. 4c). It is of note that fewer errors would be introduced during synthesis of short DNA (~ 20 nt) probe, and specificity for DNA hybridization would also increase46. More importantly, short DNA probe microarray enables target nucleic acids detection with enhanced resolution. While the sensitivity of nucleic acids detection was highly dependent on the length of the probe, and short DNA probe was always inferior to long DNA probe in terms of sensitivity46.

**Supplementary Discussion 7. Specificity improvement of clinical samples with low viral copies**

1 μL sample is sufficient for SARS-CoV-2 viral RNA detection and lineages differentiation in FEMMAN-RPA assay. While when the template in sample was too low (mainly manifested when template < 10 copies) to reach the fluorescence threshold for scanner detection, it was difficult to distinguish the lineages of amplified sample. We further increased the amount of sample used in amplification system from 1 μL to 12.5 μL (for extracted RNA) and 2.5 μL to 12.5 μL (thermal lysed clinical sample) by expanding the RPA system from 10 μL to 50 μL, and replaced the unnecessary ingredient like DNase/RNase free water by sample. The following digestion system by Lambda Exonuclease was expanded to 100 μL and then diluted to 150 μL with 10 × TE buffer and 4 M NaCl solution before loading onto the FEMMAN chip. By increasing the sample used in amplification system, the amplicon would reach the fluorescence threshold for lineage discrimination (see Data S10).

**Supplementary Discussion 8. Near-infrared fluorescence enhancement (NIR-FE) of fluorophores on plasmonic substrate**

Wide field fluorescence imaging was performed to explore the mechanism of NIR-FE on the plasmonic gold substrate. Cy5 fluorescence labeling was applied to both the DNA microarray on the plasmonic gold substrate and quartz substrate for wide field fluorescence imaging. A quartz substrate was used instead of a glass substrate due to the low auto-fluorescence background of quartz which facilitated Cy5 detection. Cy5 labeled DNA microarray detection on both the plasmonic substrate and the quartz slide showed good linearity through fluorescence imaging as evidenced by the sequence of images at progressively lower DNA target concentrations, which depicted the initial Cy5 density and fluorescence intensity immediately after illumination with the 658 nm laser (Extended Data Fig. 9a-f). At low DNA target concentrations (< 20 pM), single Cy5 blinking events (Extended Data Fig. 9i) was observed on both the plasmonic and quartz substrates (Extended Data Fig. 9b) which entailed that a proportion of the observed spots arose from single Cy5 molecules. The area density of Cy5 fluorophores was counted to be similar within the 658 nm laser spot size immediately after illumination on both the plasmonic substrate and the quartz substrate when an identical DNA target solution (20 pM) was used (Extended Data Fig. 9g, h). This reinforced that the enhancement did not arise from disparate fluorophore densities on the plasmonic and quartz substrates. The fluorescence of single Cy5 molecule on the plasmonic substrate was found to be at nearly a 50-fold higher intensity than single Cy5 molecules on the quartz slide (Extended Data Fig. 9j, k), demonstrating that the plasmonic gold enhanced the near-infrared fluorescence of single fluorophore. Furthermore, the enhancement of single Cy5 molecule from wide field imaging matched closely with the ensemble Cy5 enhancement levels observed with the microarray scanner (40 ×). The capability of enhancing single molecule fluorescence emission enabled fluorescence to be detected at ultralow fluorophore densities of 20 fM and effectively boosted the DNA detection dynamic range and sensitivity. The NIR-FE made the linear range broader since the background was similar by laser scanning. This hypothesis was validated by wide field fluorescence imaging of Cy5 fluorescence for different DNA target concentrations.

Supplementary Table 1. RPA primers used in this study

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Name** | **Target Site** | **Sequence (5' to 3')** | | **Figures** |
| Y144-RPA-PF | G142-Y145 | 5’-biotin-AAGTCTGTGAATTTCAATTTTGTAATGATCCAT-3’ | Fig. 1, 3, 4, S5, S6 | |
| Y144-RPA-PR | G142-Y145 | 5’-phosphate-CTGAACTCACTTTCCATCCAACTTTTGTTGTTT-3’ | Fig. 1, 3, 4, S5, S6 | |
| K417-RPA-PF | K417 | 5’-biotin-TTAGAGGTGATGAAGTCAGACAAATCGCTCCAG-3’ | Fig. 1, 3, 4, S5, S6 | |
| K417-RPA-PR | K417 | 5’-phosphate-CTGTAAAATCATCTGGTAATTTATAATTATAAT-3’ | Fig. 1, 3, 4, S5, S6 | |
| L452-RPA-PF | L452 | 5’-biotin-AATTCTAACAATCTTGATTCTAAGGTTGGTGGT-3’ | Fig. 1, 3, 4, S5, S6 | |
| L452-RPA-PR | L452 | 5’-phosphate-CTCAAAAGGTTTGAGATTAGACTTCCTAAACAA-3’ | Fig. 1, 3, 4, S5, S6 | |
| N501-RPA-FP2 | N501 | 5’-biotin-AGGTTTTAATTGTTACTTTCCTTTACAATCATA-3’ | Fig. 2, S3, S5, S8 | |
| N501-RPA-RP2 | N501 | 5’-phosphate-GTACTACTACTCTGTATGGTTGGTAACCAACAC-3’ | Fig. 2, S3, S5, S8 | |
| N501-RPA-FP1 | N501 | 5’-biotin-GGTGTTGAAGGTTTTAATTGTTACTTTCCTTTACAATC-3’ | Fig. S8 | |
| N501-RPA-RP1 | N501 | 5’-phosphate-TTTAGGTCCACAAACAGTTGCTGGTGCATGTAGAAGTT-3’ | Fig. S8 | |

Supplementary Table 2. FEMMAN probes used in this study

|  |  |  |  |
| --- | --- | --- | --- |
| **Name** | **Target Site** | **Sequence (5' to 3')** | **Figures** |
| Y144Y-probe | Y144Y | 5’-HS-aaaaaaaaaaTTTTTGTGGTAATAAACACC-3’ | Fig. 1, 3, 4, S5, S6 |
| Y144del-probe | Y144del | 5’-HS-aaaaaaaaaaTTTTTGTGGTAAACACCCAA-3’ | Fig. 1, 3, 4, S5, S6 |
| Y144YS145N-probe | Y144SY145N | 5’-HS-aaaaaaaaaaTGGTTAGAAGTAACACCCAA-3’ | Fig. 1, 3, 4, S5, S6 |
| G142D143-145del-probe | G142D143-145del | 5’-HS-aaaaaaaaaaGTTTTTGTGGTCCAAAAATG-3’ | Fig. 1, 3, 4, S5, S6 |
| K417K-probe | K417K | 5’-HS-aaaaaaaaaaAATCAGCAATCTTTCCAGTT-3’ | Fig. 1, 3, 4, S5, S6 |
| K417N-probe | K417N | 5’-HS-aaaaaaaaaaAATCAGCAATATTTCCAGTT-3’ | Fig. 1, 3, 4, S5, S6 |
| K417T-probe | K417T | 5’-HS-aaaaaaaaaaAATCAGCAATCGTTCCAGTT-3’ | Fig. 1, 3, 4, S5, S6 |
| L452L-probe | L452L | 5’-HS-aaaaaaaaaaCAATCTATACAGGTAATTAT-3’ | Fig. 1, 3, 4, S5, S6 |
| L452R-probe | L452R | 5’-HS-aaaaaaaaaaCAATCTATACCGGTAATTAT-3’ | Fig. 1, 3, 4, S5, S6 |
| L452Q-probe | L452Q | 5’-HS-aaaaaaaaaaCAATCTATACTGGTAATTAT-3’ | Fig. 1, 3, 4, S5, S6 |
| N501N-probe | N501N | 5’-HS-aaaaaaaaaaACACCATTAGTGGGTTGGAA-3’ | Fig. 2, S1, S3, S5, S8 |
| N501Y-probe | N501Y | 5’-HS-aaaaaaaaaaACACCATAAGTGGGTTGGAA-3’ | Fig. 2, S3, S8 |
| PTC probe | PTC | 5’-HS-aaaaaaaaaaAAGGTTGGGTGATTACCACA-biotin-3’ | Fig. 1, 3, 4, S5, S6 |
| NTC probe | H1N1 | 5’-HS-aaaaaaaaaaTCGAATAGTCTGACTACAACT-3’ | Fig. 1, 2, 3, 4, S5, S6 |
| Probe A | miRNA-21 | 5’-HS-TCAACATCAGTCTGATAAGCTA-3’ | Fig. S2, S4 |
| Probe B | miRNA-155 | 5’-HS-ACCCCTATCACGATTAGCATTAA-3’ | Fig. S2, S4 |
| Probe C | let-7a | 5’-HS-AACTATACAACCTACTACCTCA-3’ | Fig. S2, S4 |
| Probe D | miRNA-126 | 5’-HS-CGCATTATTACTCACGGTACGA-3’ | Fig. S2, S4 |
| Probe C-2 mismatches | let-7b | 5’-HS-AACCACACAACCTACTACCTCA-3’ | Fig. S4 |
| Probe C-1 mismatches | let-7c | 5’-HS-AACCATACAACCTACTACCTCA-3’ | Fig. S4 |
| Non-specific probe | miRNA-29a | 5’-HS-TAACCGATTTCAGATGGTGCTA-3’ | Fig. S4 |
| Non-specific probe | miRNA-92 | 5’-HS-ACAGGCCGGGACAAGTGCAATA-3’ | Fig. S4 |
| Non-specific probe | miRNA-93 | 5’-HS-CTACCTGCACGAACAGCACTTTG-3’ | Fig. S4 |
| Non-specific probe | miRNA-141 | 5’-HS-TCCAACACTGTACTGGAAGATG-3’ | Fig. S4 |
| Non-specific probe | miRNA-195 | 5’-HS-GCCAATATTTCTGTGCTGCTA-3’ | Fig. S4 |
| Non-specific probe | miRNA-210 | 5’-HS-TCAGCCGCTGTCACACGCACAG-3’ | Fig. S4 |
| Non-specific probe | miRNA-221 | 5’-HS-GAAACCCAGCAGACAATGTAGCT-3’ | Fig. S4 |
| Non-specific probe | miRNA-222 | 5’-HS-ACCCAGTAGCCAGATGTAGCT-3’ | Fig. S4 |
| Non-specific probe | miRNA-483 | 5’-HS-CTCCCTTCTCTTCTCCCGTCTT-3’ | Fig. S4 |
| Type 1-probe | N501N | 5’-ACACCATTAGTGGGTTGGAAaaaaaaaaaa-SH-3’ | Fig. S8 |
| Type 3-probe | N501N | 5’-HS-aaaaaaaaaaaaaaaaaaaaaaaaaaaaaaACACCATTAGTGGG  TTGGAA-3’ | Fig. S8 |

Supplementary Table 3. Mutation sites in SARS-CoV-2 variants

|  |  |  |
| --- | --- | --- |
| **Name** | **Sample types** | **Mutation sites** |
| SARS-CoV-2 wild type | Synthetic RNA & lentivirus | - |
| SARS-CoV-2 B.1.1.7 (Alpha variant) | Lentivirus | Δ69-70, **Δ144**, N501Y, A570D, D614G, P681H, T716I, S982A, D1118H |
| SARS-CoV-2 B.1.351 (Beta variant) | Lentivirus | L18F, D80A, D215G，Δ242-244, **K417N**, E484K, N501Y, D614G, A701V |
| SARS-CoV-2 P.1 (Gamma variant) | Lentivirus | L18F, T20N, P26S, D138Y, R190S, **K417T**, E484K, N501Y, D614G, H655Y, T1027I, V1176F |
| SARS-CoV-2 B.1.617.2 (Delta variant) | Synthetic RNA & lentivirus | T19R, G142D, Δ156, Δ157, R158G, **L452R**, T478K, D614G, P681R, D950N |
| SARS-CoV-2 C.37 (Lambda variant) | Lentivirus | G75V, T76I, Δ246/253, **L452Q**, F490S, D614G, T859N |
| SARS-CoV-2 B.1.621 (Mu variant) | Lentivirus | T95I, **Y144S, Y145N**, R346K, E484K, N501Y, D614G, P681H, D950N |
| SARS-CoV-2 B.1.1.529 (Omicron variant) | Lentivirus | A67V Δ69 Δ70 T95I **G142D Δ143-145** Δ211 ins214EPE G339D S371L S373P S375F **K417N** N440K G446S S477N T478K E484A Q493R G496S Q498R N501Y Y505H T547K D614G H655Y N679K P681H N764K D796Y N856K Q954H N969K L981F |

Supplementary Table 4. DNA and RNA targets used in this study

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Name** | **Sample types** | **Target Site** | **Sequence (5' to 3')** | **Figures** |
| N501N-target-20 nt | Synthetic DNA | N501 | 5’-biotin-TTCCAACCCACTAATGGTGT-3’ | Fig. S1, S8 |
| Target A | Synthetic DNA | miRNA-21 | 5’-biotin-TAGCTTATCAGACTGATGTTGA-3’ | Fig. S2, S4 |
| Target B | Synthetic DNA | miRNA-155 | 5’-biotin-TTAATGCTAATCGTGATAGGGGT-3’ | Fig. S2, S4 |
| Target C | Synthetic DNA | let-7a | 5’-biotin-TGAGGTAGTAGGTTGTATAGTT-3’ | Fig. S2, S4 |
| Target D | Synthetic DNA | miRNA-126 | 5’-biotin-TCGTACCGTGAGTAATAATGCG-3’ | Fig. S2, S4 |
| Y144Y-target in viral RNA | Synthetic RNA & lentivirus | Y144Y | ...*AAGUCUGUGAAUUUCAAUUUUGUAAUGAUCCAU*UUUUG**GGUGUUUAUUACCACAA*AAA****CAACAAAAGUUGGAUGGAAAGUGAGUUCAG*… | Fig. 1, 3, S5, S6 |
| Y144del-target in viral RNA | lentivirus | Y144del | ...*AAGUCUGUGAAUUUCAAUUUUGUAAUGAUCCAU*UU**UUGGGUGUUUACCACAA*AAA****CAACAAAAGUUGGAUGGAAAGUGAGUUCAG*… | Fig. 1, 3, S5, S6 |
| Y144SY145N-target in viral RNA | lentivirus | Y144SY145N | ...*AAGUCUGUGAAUUUCAAUUUUGUAAUGAUCCAU*UU**UUGGGUGUUACUUCUAACCA**CAA*AAACAACAAAAGUUGGAUGGAAAGUGAGUUCAG*… | Fig. 1, 3, S5, S6 |
| G142D143-145del-target in viral RNA | lentivirus | G142D143-145del | ...*AAGUCUGUGAAUUUCAAUUUUGUAAUGAUC****CAU*UUUUGGACCACAA*AAAC****AACAAAAGUUGGAUGGAAAGUGAGUUCAG*… | Fig. 1, 3, S5, S6 |
| K417K-target in viral RNA | Synthetic RNA & lentivirus | K417K | ...*UUAGAGGUGAUGAAGUCAGACAAAUCGCUCCAG*GGCA**AACUGGAAAGAUUGCUG*AUU****AUAAUUAUAAAUUACCAGAUGAUUUUACAG*… | Fig. 1, 3, S5, S6 |
| K417N-target in viral RNA | lentivirus | K417N | ...*UUAGAGGUGAUGAAGUCAGACAAAUCGCUCCAG*GGCA**AACUGGAAAUAUUGCUG*AUU****AUAAUUAUAAAUUACCAGAUGAUUUUACAG*… | Fig. 1, 3, S5, S6 |
| K417T-target in viral RNA | lentivirus | K417T | ...*UUAGAGGUGAUGAAGUCAGACAAAUCGCUCCAG*GGCA**AACUGGAACGAUUGCUG*AUU****AUAAUUAUAAAUUACCAGAUGAUUUUACAG*… | Fig. 1, 3, S5, S6 |
| L452L-target in viral RNA | Synthetic RNA & lentivirus | L452L | ...*AAUUCUAACAAUCUUGAUUCUAAGGUUGGUGGU*AAUU**AUAAUUACCUGUAUAGA*UUG****UUUAGGAAGUCUAAUCUCAAACCUUUUGAG*… | Fig. 1, 3, S5, S6 |
| L452R-target in viral RNA | Synthetic RNA & lentivirus | L452R | ...*AAUUCUAACAAUCUUGAUUCUAAGGUUGGUGGU*AAUU**AUAAUUACCGGUAUAGA*UUG****UUUAGGAAGUCUAAUCUCAAACCUUUUGAG*… | Fig. 1, 3, S5, S6 |
| L452Q-target in viral RNA | lentivirus | L452Q | ...*AAUUCUAACAAUCUUGAUUCUAAGGUUGGUGGU*AAUU**AUAAUUACCAGUAUAGA*UUG****UUUAGGAAGUCUAAUCUCAAACCUUUUGAG*… | Fig. 1, 3, S5, S6 |
| N501N-target in viral RNA for Type 5 | Synthetic DNA | N501N | ...*GGUGUUGAAGGUUUUAAUUGUUACUUUCCUUUACAAUC*AUAUGGU**UUCCAACCCACUAAUG*GUGU****UGGUUACCAACCAUACAGAGUAGUAGUAC*… | Fig. S8 |
| N501N-target in viral RNA for Type 6 | Synthetic DNA | N501N | ...*AGGUUUUAAUUGUUACUUUCCUUUACAAUCAUA*UGGU**UUCCAACCCACUAAUGGUGU**UGGUUACCAACCAUACAGAGUAGUAGUACUUUCUUUUG*AACUUCUACAUGCACCAGCAACUGUUUGUGGACCUAAA*… | Fig. S8 |
| N501N-target in viral RNA for Type 7 | Synthetic DNA | N501N | ...*AGGUUUUAAUUGUUACUUUCCUUUACAAUCAUA*UGGU**UUCCAACCCACUAAUG*GUGU****UGGUUACCAACCAUACAGAGUAGUAGUAC*… | Fig. 2, S3, S5, S8 |
| N501N-target in viral RNA for Type 8 | Synthetic DNA | N501N | ...*GGUGUUGAAGGUUUUAAUUGUUACUUUCCUUUACAAUC*AUAUGGU**UUCCAACCCACUAAUGGUGU**UGGUUACCAACCAUACAGAGUAGUAGUACUUUCUUUUG*AACUUCUACAUGCACCAGCAACUGUUUGUGGACCUAAA*… | Fig. S8 |

Supplementary Table 5. Table of qPCR primer/probe sequences

|  |  |  |  |
| --- | --- | --- | --- |
| **Name** | **Target Site** | **Sequence (5' to 3')** | **Figures** |
| N501-PCR-FP | N501 | 5’-GCATGTAGAAGTTCAAAAGAAAGT-3’ | Fig. 3, 4 |
| N501-PCR-RP | N501 | 5’-TCCTTTACAATCATATGGTTTCCA-3’ | Fig. 3, 4 |
| N501N-probe | N501N | 5’-VIC-CACTAATGGTGTTGGTTACCAACCA-MGB-3’ | Fig. 3, 4 |
| N501Y-probe | N501Y | 5’-FAM-CACTTATGGTGTTGGTTACCAACCA-MGB-3’ | Fig. 3, 4 |
| S protein-PCR-FP | S protein | 5’-ATGTTTGTTTTTCTTGTTTTATTGCCACTAGTC-3’ | Fig. 4 |
| S protein-PCR-RP | S protein | 5’-TGATAAAGAACAGCAACCTGGTTAGAAGTATTT-3’ | Fig. 4 |

**Reference:**

38 Tabakman, S. M. *et al.* Plasmonic substrates for multiplexed protein microarrays with femtomolar sensitivity and broad dynamic range. *Nat. Commun.* **2**, 466, doi:10.1038/ncomms1477 (2011).

39 Rauzan, B. *et al.* Kinetics and thermodynamics of DNA, RNA, and hybrid duplex formation. *Biochemistry* **52**, 765-772, doi:10.1021/bi3013005 (2013).

40 Sambrook, M. R. G. a. J. Analysis of DNA by Southern Blotting. *Cold Spring Harbor Laboratory Press* (2021).

41 Qi, B. *et al.* Tuned near infrared fluorescent hyaluronic acid conjugates for delivery to pancreatic cancer for intraoperative imaging. *Theranostics* **10**, 3413-3429, doi:10.7150/thno.40688 (2020).

42 Wang, Y., Li, X., Xi, D. & Wang, X. Visual detection of Fusarium proliferatum based on asymmetric recombinase polymerase amplification and hemin/G-quadruplex DNAzyme. *RSC Advances* **9**, 37144-37147, doi:10.1039/c9ra05709a (2019).

43 Georgescu, R. E. *et al.* Mechanism of asymmetric polymerase assembly at the eukaryotic replication fork. *Nat. Struct. Mol. Biol.* **21**, 664-670, doi:10.1038/nsmb.2851 (2014).

44 Higgins, M. *et al.* PrimedRPA: primer design for recombinase polymerase amplification assays. *Bioinformatics* **35**, 682-684, doi:10.1093/bioinformatics/bty701 (2019).

45 Wu, H. *et al.* A Recombinase Polymerase Amplification and Lateral Flow Strip Combined Method That Detects Salmonella enterica Serotype Typhimurium With No Worry of Primer-Dependent Artifacts. *Front. Microbiol.* **11**, 1015, doi:10.3389/fmicb.2020.01015 (2020).

46 Miller, M. B. & Tang, Y. W. Basic concepts of microarrays and potential applications in clinical microbiology. *Clin. Microbiol. Rev.* **22**, 611-633, doi:10.1128/CMR.00019-09 (2009).