# Supporting Information

# Tetrahedral DNA Nanostructure-decorated Electrochemical Platform for Simple and Ultrasensitive EGFR Genotyping of Plasma ctDNA

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Table of the sequences used. Photograph of SPE used. Additional data and figures of the assay.

**Table S1.** Sequences of the DNA oligonucleotides employed in this study.

| Name | Sequence (from 5' to 3') | Remarks |
|---|---|---|
| Tetra-B | SH-$C_6$ - TATCACCAGGCAGTTGACAGTGTAGCAAG CTGTAATAGATGCGAGGGTCCAATAC | The sequence to form the scaffold of the tetrahedron |
| Tetra-C | SH-$C_6$ - TCAACTGCCTGGTGATAAAACGACACTACG TGGGAATCTACTATGGCGGCTCTTC | The sequence to form the scaffold of the tetrahedron |
| Tetra-D | SH-$C_6$ – TTCAGACTTAGGAATGTGCTTCCCACGTAG TGTCGTTTGTATTGGACCCTCGCAT | The sequence to form the scaffold of the tetrahedron |
| Tetra-2A19 | ACATTCCTAAGTCTGAAACATTACAGCTTG CTACACGAGAAGAGCCGCCATAGTATTTTT TTTTTGGATCCCAGAAGGTGAGAAAGTTAA AATTCCCGT | The sequence to form the scaffold of the tetrahedron with a pendant probe complementary to exon 19 target |
| Tetra-2A21 | ACATTCCTAAGTCTGAAACATTACAGCTTG CTACACGAGAAGAGCCGCCATAGTATTTTT TTTTTAAACACCGCAGCATGTCAAGATCAC AGATTTTGG | The sequence to form the scaffold of the tetrahedron with a pendant probe complementary to exon 21 target |
| Tetra-3A | TTCCCCACGAGGGCTTCAACTCTATTTTTTT TTTACATTCCTAAGTCTGAAACATTACAGCT TGCTACACGAGAAGAGCCGCCATAGTA | The sequence to form the scaffold of the tetrahedron with a pendant probe complementary to the assistant probe |
| 19ass | TAGAGTTGAAGCCCTCGTGGGGAATTGGA TCCCAGAAGGTGAGAAAGTTAAAATTCCC GT | Complementary to exon 19 and Tetra-3A |
| 21ass | TAGAGTTGAAGCCCTCGTGGGGAATTAAA CACCGCAGCATGTCAAGATCACAGATTTTG G | Complementary to exon 21 and Tetra-3A |
| 19AntiT | TTAACGTCTTCCTTCTCTCTCTGTCATAGGG ACTCTGGATCCCAGAAGGTGAGAAAGTTA AAATTCCCGTCGCTATCAAAACATCTCCGA AAGCCAACA | Anti-target sequence of exon 19 |
| 21AntiT | GTCGCTTGGTGCACCGCGACCTGGCAGCC AGGAACGTACTGGTGAAAACACCGCAGCA TGTCAAGATCACAGATTTTGGGCGGACCA AACTGCTGGGTG | Anti-target sequence of exon 21, the red nucleotide is adjusted to fit 21MR |
| 19F | TGCCAGTTAACGTCTTCCTTC | Forward primer |
| 19F2 | TTGCCAGTTAACGTCTTCCTTC | An extra nucleotide was added to fix the Tm. Labeled in red. |

| | | |
|---|---|---|
| 19WR | Biotin-GGCTTTCGGAGATGTTGCTTCTC | Complementary to wild type |
| 19MR1 | Biotin-TGTTGGCTTTCGGAGATGTTTTG | Complementary to mutated type. Three nucleotides were appended at the split. Labeled in red. |
| 19MR2 | Biotin-GGCTTTCGGAGATGTTTTGATAG | Complementary to mutated type. Seven nucleotides were appended at the split. Labeled in red. |
| 21F | GCATGAACTACTTGGAGGAC | Forward primer |
| 21F2 | GGCATGAACTACTTGGAGGAC | An extra nucleotide was added to fix the Tm. Labeled in red |
| 21WR | Biotin-CACCCAGCAGTTTGGTCA | Complementary to wild type |
| 21MR1 | Biotin-CACCCAGCAGTTTGGTCC | Complementary to mutated type, with an extra mismatch at -2 position. Labeled in red. |
| 21MR2 | Biotin-CACCCAGCAGTTTGGCCC | Complementary to mutated type |


**Figure S1.** Photograph of SPE used in this platform.

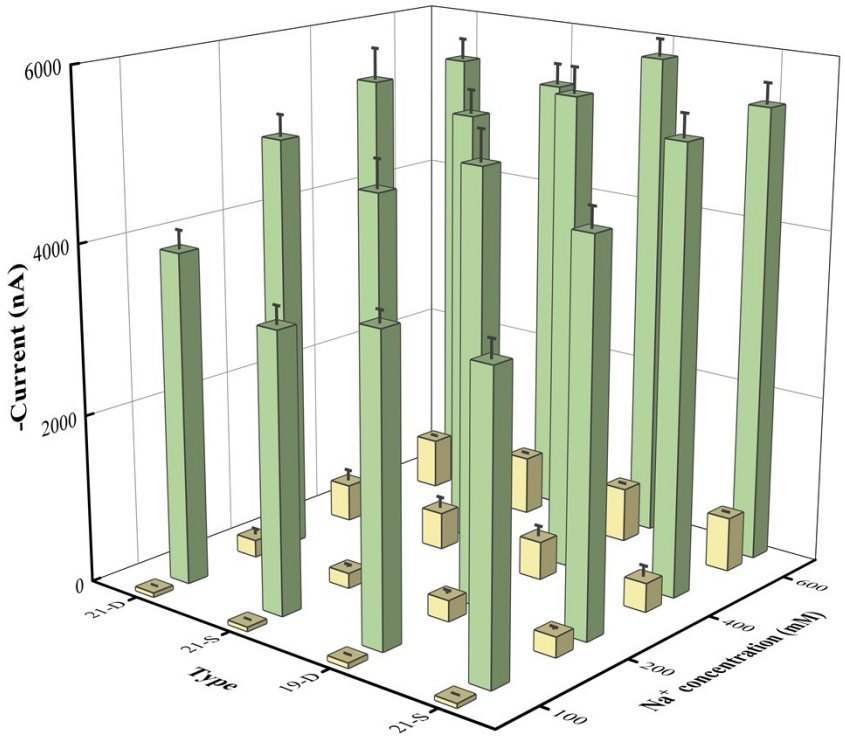


**Figure S2.** Optimal conditions for $Na^+$ concentration.

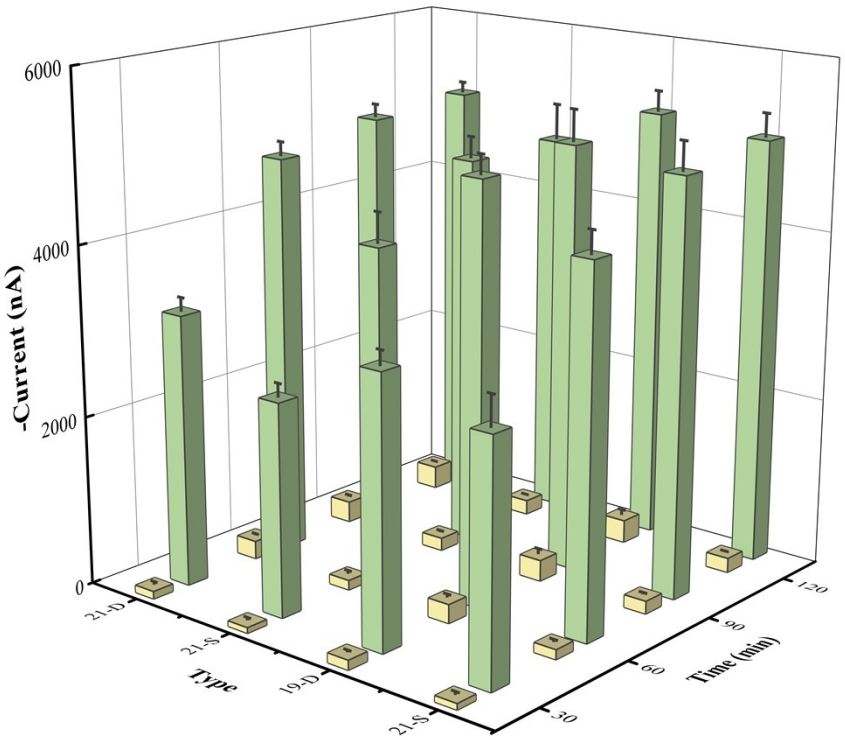


**Figure S3.** Optimal conditions for hybridization time.

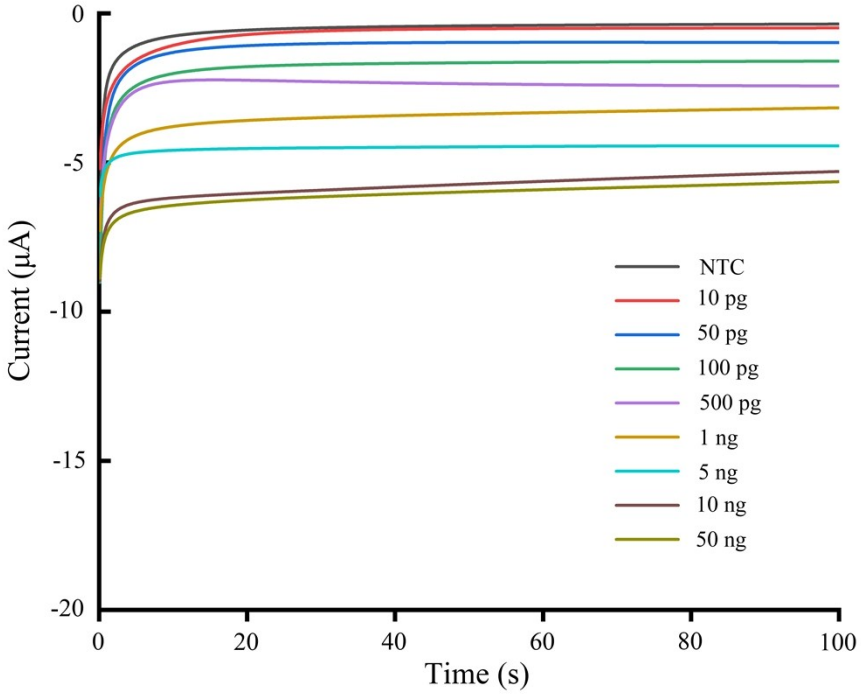


**Figure S4.** Current values of different amount of 19del DNA input. The signal gradually rises as more DNA is added.

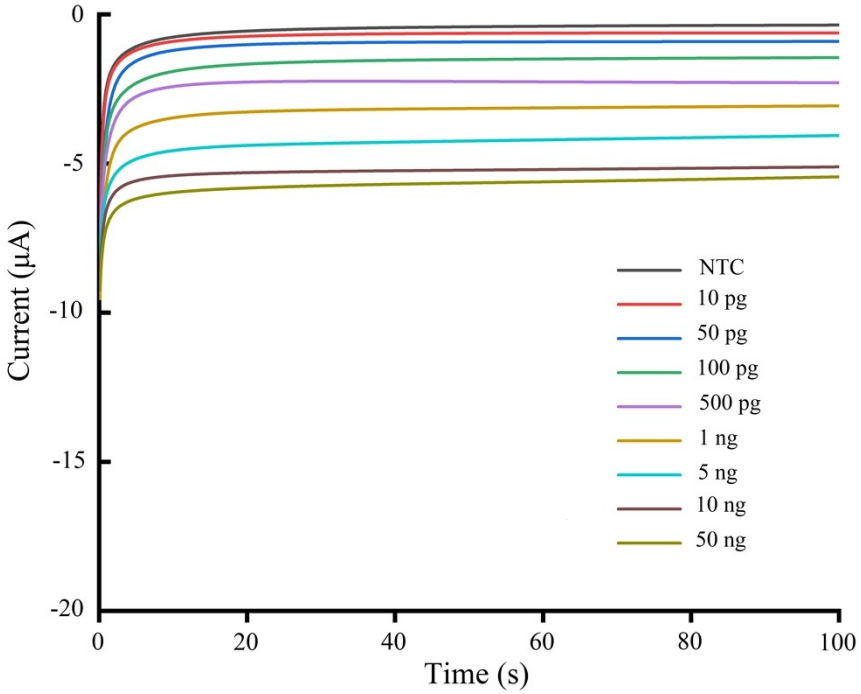


**Figure S5.** Current values of different amount of L858R DNA input. The signal gradually rises as more DNA is added.

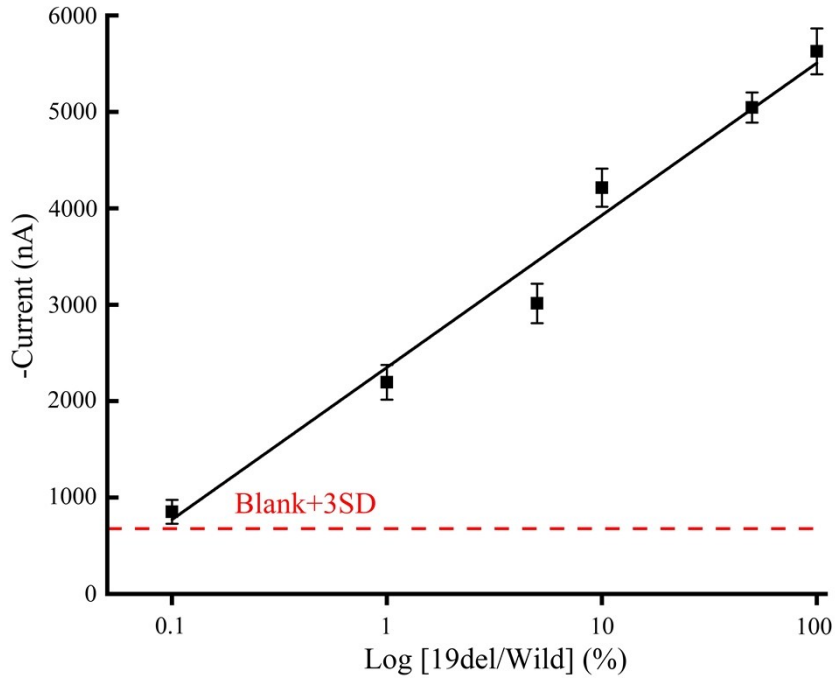


**Figure S6.** Chronoamerometric currents of the different percentages of 19del in the mixture.

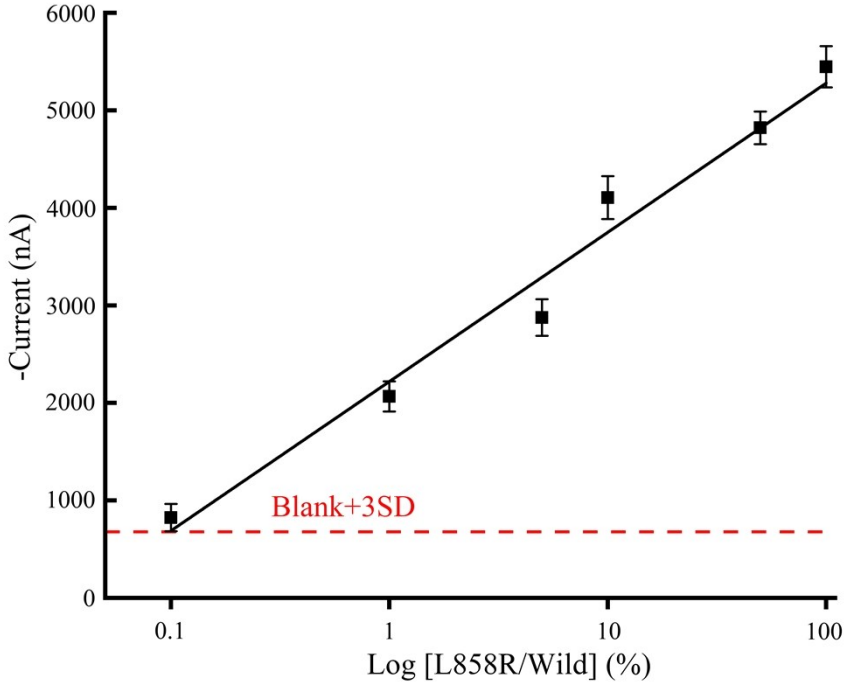


**Figure S7.** Chronoamerometric currents of the different percentages of L858R in the mixture.

**Table S2.** The reproducibility of the platform.

| No. | 1 | 2 | 3 | SD | Average | RSD |
|---|---|---|---|---|---|---|
| Current (nA) | 7523 | 7674 | 7390 | 142.1 | 7529 | 1.89% |

**Table S3.** The list of abbreviations in the main text.

| Full phrase | Abbreviations |
|---|---|
| Non-small cell lung cancer | NSCLC |
| Epidermal growth factor receptor tyrosine kinase inhibitors | EGFR-TKIs |
| Epidermal growth factor receptor | EGFR |
| Circulating tumor DNA | ctDNA |
| Next-generation sequencing | NGS |
| Amplification refractory mutation system | ARMS |
| Droplet digital PCR | dd-PCR |
| affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free, and deliverable to end-users | ASSURED |
| Point-of-care testing | POCT |
| Hybridization chain reaction | HCR |
| Rolling circle amplification | RCA |
| Linear-After-The-Exponential | LATE |
| Single-stranded DNA | ssDNA |
| Screen-printed electrodes | SPE |
| 3,3’,5,5’-tetramethylbenzidine | TMB |
| Avidin-horseradish peroxidase | HRP |
| Cyclic voltammetry | CV |
| Fetal bovine serum | FBS |
| Three standard deviations | 3SD |
| Non-template control | NTC |