

# Integrated Approach from Sample-to-Answer for Grapevine Varietal Identification on a Portable Graphene Sensor Chip

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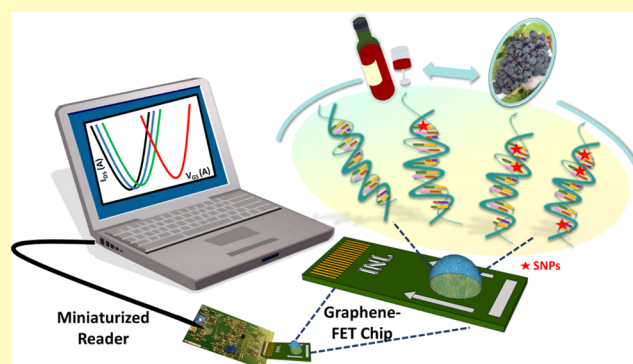
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Supporting Information

**ABSTRACT:** Identifying grape varieties in wine, related products, and raw materials is of great interest for enology and to ensure its authenticity. However, these matrices' complexity and low DNA content make this analysis particularly challenging. Integrating DNA analysis with 2D materials, such as graphene, offers an advantageous pathway toward ultrasensitive DNA detection. Here, we show that monolayer graphene provides an optimal test bed for nucleic acid detection with single-base resolution. Graphene's ultrathinness creates a large surface area with quantum confinement in the perpendicular direction that, upon functionalization, provides multiple sites for DNA immobilization and efficient detection. Its highly conjugated electronic structure, high carrier mobility, zero-energy band gap with the associated gating effect, and chemical inertness explain graphene's superior performance. For the first time, we present a DNA-based analytic tool for grapevine varietal discrimination using an integrated portable biosensor based on a monolayer graphene field-effect transistor array. The system comprises a wafer-scale fabricated graphene chip operated under liquid gating and connected to a miniaturized electronic readout. The platform can distinguish closely related grapevine varieties, thanks to specific DNA probes immobilized on the sensor, demonstrating high specificity even for discriminating single-nucleotide polymorphisms, which is hard to achieve with a classical end-point polymerase chain reaction or quantitative polymerase chain reaction. The sensor was operated in ultralow DNA concentrations, with a dynamic range of 1 aM to 0.1 nM and an attomolar detection limit of  $\sim 0.19$  aM. The reported biosensor provides a promising way toward developing decentralized analytical tools for tracking wine authenticity at different points of the food value chain, enabling data transmission and contributing to the digitalization of the agro–food industry.

**KEYWORDS:** wine authenticity, grapevine, DNA sensor, graphene, field-effect transistor, SNP



Wine has gained popularity around the whole globe, with the prediction of a global market revenue from 2012 to 2025, valued at 340.8 billion USD, which is expected to rocket to nearly 423 billion USD by the end of 2023,<sup>1,2</sup> and it is estimated to expand continuously. Wine offers a great diversity and depth of sensory characteristics, depending mainly on the grape variety, climate conditions, production technology, and vintage. These factors dictate the unique quality and, ultimately, the value of wine in the commercial market, making it an appealing collector target and even an investment asset. Due to its solid market reputation, wine is one of the products with a higher risk of being the target of fraudulent practices. Such practices typically involve intrinsic, such as dilution and adulteration using alcohol, colorants, or flavoring substances, and extrinsic such as fraudulent misrepresentation of the cultivar and geographic origin properties.<sup>3</sup> For instance, according to Romano et al.,<sup>4</sup> wine accounts for almost 90% of the total value of seized agro–food products in Italy during

2007–2015, by far being the dominant fraud-affected commodity. Their analysis revealed an enormous vulnerability of this food value chain and an impact of the potential wine fraud scandal on the Italian economy, with an estimated 406 million EUR loss of total output, leading as well to a significant loss of jobs, considering the accounted value of irregular (falsified) products. Apart from tremendous economic effects, loss of reputation, and customer trust at stake, there are also health, ethical, and religious aspects concerned with food adulteration that make it an alarming worldwide issue.

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The protection of agricultural commodities and products has strictly prevailed in the European Union (EU) legislative frameworks, including certain products associated with a specific origin area and specific methods of production by conferring geographical indication (GI) recognition, namely, protected designation of origin (PDO), protected geographical indication (regulation (EU) no 1308/2013), and GI of spirit drinks and aromatized wines.<sup>5,6</sup> Wines under these quality schemes should be produced according to a specific legislative framework dictating particular grapevine varieties, cultivation, vinification methods, aging, physical/chemical, and organoleptic characteristics. Moreover, the highly valued wines belonging to a specific PDO are typically produced by a single variety (monovarietal wines).<sup>7</sup> Nevertheless, these austere measures are still inadequate to combat fraud or wine mislabeling, leading to unfair market competitiveness. While many fraudulent practices can quickly be revealed by routine laboratory analysis, the differentiation between wines of different grape varieties remains a challenge, and, in general, advanced instrumental analysis is required for this purpose. There are different targets in any analytical detection in food control. Genomic methods employ nuclear or mitochondrial, as well as chloroplast DNA to generate genetic profiles. Proteomics characterizes food and food products through the analysis of proteins and peptides. Metabolomics provides deep insights into the overall composition of food products, for example, by liquid chromatography–mass spectrometry (LC–MS).

In contrast, isotopologomics can provide the analysis of isotopic ratios and rare-earth elements {e.g., SNIF–NMR or elemental analysis–isotope ratio mass spectrometry (EA–IRMS) or infrared mass spectroscopy (IR–MS) and sensory analyses [electronic nose and gas chromatography (GC)]}.<sup>8</sup> These standard approaches typically come with complex statistical analyses of a big data set from the defined region, considering several production years and varieties to develop a reliable database. The inconsistencies of the results obtained by these analytical assays (e.g., proteins and metabolites such as phenolic/volatile compounds) due to environmental conditions and processing protocols make molecular DNA-based methods the preferred method when dealing with grapevine varietal identification. The genomic fingerprinting material (DNA molecule) is highly stable under different environments and preserved despite a long-term evolution.<sup>9</sup> More importantly, the existing standard analyses in wine authenticity rarely target varietal discrimination, which could be precisely confirmed only by genetic information of the grapevine as the primary wine ingredient. Integrating analytical platforms with omics techniques has been proven beneficial in providing critical information about food composition.<sup>10</sup> Specifically, DNA-based methodologies relying on specific sequences have paved the way to prove the authenticity of raw ingredients and trace mislabeling and cross-contamination<sup>11</sup> more straightforwardly.

The study of DNA for the differentiation between plant materials at different taxonomic levels, including varietal analysis, has been widely employed due to its high specificity. *Vitis vinifera* L., or grapevine, is the main ingredient in the winemaking protocols. *Vitis vinifera* L. has grown into a wealth of highly adaptable varieties. It is reported that in Portugal alone, there are 343 varieties used in the winemaking process, while 240 are regarded as native.<sup>9,12</sup> It is noteworthy that Portugal is an essential player in the wine industry, ranking 5th

among the European countries and 11th among global producers.<sup>13</sup> Thus, analytical tools for grapevine identification and wine quality and authenticity control are urgent.

With the completion of genomic sequencing of the plant genome in 2007,<sup>14,15</sup> the identification of grapevine varieties through markers, such as single simple sequence repeats and single-nucleotide polymorphisms (SNPs), referring to the alteration at a single nucleic acid base level at a particular position in the genome, has provided a reliable alternative for varietal identification. Moreover, it is essential to note that SNPs are a robust biomarker for varietal distinction associated with quantitative trait loci<sup>16</sup> since they are predominantly biallelic,<sup>12</sup> rich in genomes with a low mutation rate, and hence, remain steady even under long time evolution.<sup>9</sup> The SNP assay is highly informative, with a high frequency of occurrence and reproducibility, as well as great automation that allows species identification and differentiation according to the target DNA (tDNA).<sup>8</sup> In particular, structural and regulatory anthocyanin biosynthetic pathway-associated genes directly influence the anthocyanin profile and have the potential for varietal determination.<sup>17,18</sup> The identification of grapevine varieties based on DNA analysis to date relies on advanced detection instrumentation combined with DNA amplification and on DNA sequencing techniques. However, discriminating varieties that have very similar sequences, differing in many cases in one single nucleotide, together with the high cost of DNA sequencing are still the main obstacle in wine authenticity control, particularly for its use by small producers or small laboratories supporting the work of farmers and wineries. In addition, the most limiting factor is the low DNA content<sup>19</sup> since physical and chemical treatments frequently lead to random breaks in the strands, reducing fragment size,<sup>20</sup> the presence of potentially interfering DNA from yeast and bacteria, and the presence of inhibitors of amplification reactions.<sup>21</sup> Nevertheless, the DNA degradation factors do not impede the application of a molecular-based authentication approach.<sup>22,23</sup> The extraction and amplification steps are required since significant differences among samples might be found if not fully optimized and tested.

With the specific detection of the grape varieties of interest, appropriate sample preparation must be used to ensure the extraction and purification of enough DNA from the grape, must, and wine samples free from a critical number of other compounds and cell debris in such complex samples. This step is of paramount importance since it can interfere with the sensing step if not performed correctly, leading to false negatives. One of the most common DNA extraction methods from precipitated wine and debris is the cetyltrimethylammonium bromide (CTAB) method which relies on cell lysis by buffer with specific active compounds.<sup>24</sup> Pereira et al., 2011, reported a DNA wine extraction method starting with a 10 mL sample that resulted in a 3 ng DNA yield by 2-propanol precipitation, enzyme treatment, phenol and chloroform extraction, and several washes.<sup>25</sup> Onache et al., 2021, showed the efficiency of DNA yield from different wine samples ranging from 18 to 85 ng/ $\mu$ L using Qiagen DNeasy Plant Kit.<sup>26</sup> It should be taken into account that not all reportedly successful methods could be applied to any wine samples. DNA amplification is frequently performed for the enrichment of the total DNA of a particular species by the use of universal primers for such species. This procedure helps avoid interference with DNA from other species, such as yeast or bacteria, present in the wine and vegetal samples. This DNA



**Figure 1.** Protocols for varietal discrimination in wine and grapevine based on DNA analysis.

amplification, commonly performed by polymerase chain reaction (PCR), must be designed and optimized carefully. However, the identification of plant varieties by PCR is highly challenging. DNA arrays are a good alternative for the specific detection of closely related varieties since using very short DNA sequences immobilized on a sensing surface allows monitoring minor differences even at the SNP level.

Among analytical detection methods in food quality and safety, biosensors have emerged as game-changing tools due to their rapidity, low cost, miniaturization, simplicity, user-friendliness, and, more importantly, specificity and sensitivity, providing an exciting alternative for the development of miniaturized tools for on-site or decentralized analysis. Mainly, among the various DNA-based systems, biosensors have emerged as an attractive and alternative method for food authenticity.<sup>8</sup> The specific detection of selected DNA markers can be achieved using various strategies, such as PCR and sequencing,<sup>27</sup> optical,<sup>28,29</sup> piezoelectric,<sup>30</sup> magnetic,<sup>31</sup> and electrochemical methods.<sup>32,33</sup> However, these detection techniques rely on label reagents, such as enzymes or fluorescent dyes, requiring strictly controlled reaction conditions. Field-effect transistors (FET) represent an attractive, direct, label-free detection approach. FETs can be readily integrated with other electronic components, such as data analyzers and signal transducers. FETs are typically functionalized with a biorecognition element that binds the target analyte in a medium and generates an electrical signal, namely, a change in conductivity or mobility.<sup>34,35</sup> Two-dimensional (2D) van der Waals (vdW) layered materials, including graphene, transition-metal dichalcogenides (TMDs), hexagonal boron nitride (hBN), and MXenes have attracted significant attention in recent years. However, it is noteworthy that graphene was the first to pioneer the further exploration of other 2D materials for biosensing technologies. Regarding large-scale production and marketing, among different types of 2D materials, only large-area graphene films and graphite oxide powders are currently available in the commercial market.<sup>36</sup> It is also noteworthy that the single-layer nature of graphene provides the optimal thinness for single-base resolution of nucleic acid detection. Furthermore, regarding the wafer-scale production as our target in this research, only single-crystal graphene films have been successfully synthesized at the wafer scale with layer control. Still, other 2D materials' fabrication, such as hBN and TMDs, is limited exclusively to single-crystal monolayer films.<sup>37</sup> Cao et al., 2022, compared graphene, MoS<sub>2</sub>, and titanium carbide MXene (Ti<sub>3</sub>C<sub>2</sub>) for nanopore-based DNA detection using molecular dynamics simulation for the translocation of four different types of single-strand DNA (ssDNA) through ionic current (*I*–*V*) analyses.<sup>38</sup> It is reported that the higher residence time of DNA bases in graphene nanopores is significantly associated with the physisorption of DNA bases on the graphene interface, and the DNA bases' physisorption strength follows this order: graphene > MXene > MoS<sub>2</sub>. In addition, graphene is a remarkable 2D material due

to its excellent electronic performance even when its surface is exposed to ambient conditions; mechanical strength; flexibility; ultimate thinness; flexible layered structures; good biocompatibility; and strong interfacial interactions with biomolecules, proteins, DNA, cells, and other biocompounds.<sup>39,40</sup> Graphene can be obtained via various processes, such as chemical vapor deposition (CVD), which lends itself to fabricating large-area, wafer-scalable devices.<sup>41</sup> Kwong Hong Tsang et al., 2019, successfully fabricated a graphene FET biosensor using monolayer graphene with a back-gate setup and chemically functionalized graphene with an antibody for exosome detection.<sup>42</sup> A printable graphene BioFET for DNA quantification utilizing the core board as the substrate and the printed circuit board (PCB) electrodes as the transistor drain, source, and gate pads was reported by Papamaththaiou et al. 2021. These and many other examples show the versatility of graphene FET sensors.<sup>43,44</sup>

This study reports varietal discrimination of Portuguese grapevines from PDO Douro through the hybridization of complementary DNA targets on the surface of a graphene field-effect transistor (GFET) chip with a portable system. The detection approach was tested with complex grapevine and monovarietal wine matrices, enabling a prominent signal differentiation for the different varieties even at SNP levels, with an outstanding attomolar range detection limit, which renders it as a unique platform for varietal differentiation to be used for wine authenticity assessment that also paves the way for DNA-based pervasive ampelographic analysis. The graphene chip was fabricated with CVD-grown monolayer graphene with a multiaarrayed sensor layout, plugged in directly to an Arduino-compatible electronic control board with output reading and connected to a laptop. Reliable detection of the hybridization of the complementary DNA target is achieved by the distinguishable difference in the Dirac voltage ( $V_{\text{Dirac}}$ ) after the interaction of the probe DNA (pDNA) with the target and the noncomplementary (NC) DNA sequences. We have demonstrated variety detection in complex grapevine samples based on the careful optimization of DNA extraction and purification and DNA amplification, followed by a highly specific and sensitive label-free DNA sequence detection using a miniaturized GFET sensor (Figure 1).

## EXPERIMENTAL SECTION

**Materials.** Chemicals and reagents, such as phosphate-buffered saline (PBS), NaCl and MgCl<sub>2</sub>, 1-dodecanethiol (DDT), ethanol-amine (ETA), and 1-pyrene butyric acid succinimidyl ester (PBSE) and other solvents, including acetone and ethyl acetate, were purchased from Sigma-Aldrich. In all the experimental stages, the deionized water from a Milli-Q system was used (resistivity at 25 °C = 18.2 MΩ cm). For graphene growth, a highly purified copper foil (>99.99%) was purchased from Alfa Aesar (Ward Hill, MA, USA) or Goodfellow GmbH (Hamburg, Germany). The DNA used in the hybridization study was from specific regions from the flavanone-3-hydroxylase (F3H) gene of some selected Portuguese grapevine varieties, as reported elsewhere.<sup>9</sup> Specific sequences, including 35-mer



Table 1. DNA Sequences Used in This Study

| DNA name                    | sequence                                          |
|-----------------------------|---------------------------------------------------|
| probe DNA (pDNA)            | 5'-C6-amine-GCGAAAGCTGAAGCTAATCTTTTCTTTGTCTTTG-3' |
| full complementary (FC) DNA | 5'-CAAAGACAAAGAAAAGATTAGCTTCAGCCTTTC GC-3'        |
| 1 mismatch (1 SNP)          | 5'-CAAAGACAAAGAAAAGATTAGCTTCAGCCTATC GC-3'        |
| 2 mismatches (2 SNPs)       | 5'-CAAAGACAAAGAAAAGATTAGCTTCAGCCAATC GC-3'        |
| 3 mismatches (3 SNPs)       | 5'-CAAAATACAAAGAAAAGATTAGCTTCAGCCAATC GC-3'       |
| noncomplementary (NC) DNA   | 5'-GGACCTTTCGTGGTGAATCTTGGAGACCATGGACA-3'         |

Scheme 1. Schematic Illustration of DNA Detection Using the Fabricated GFET Chip

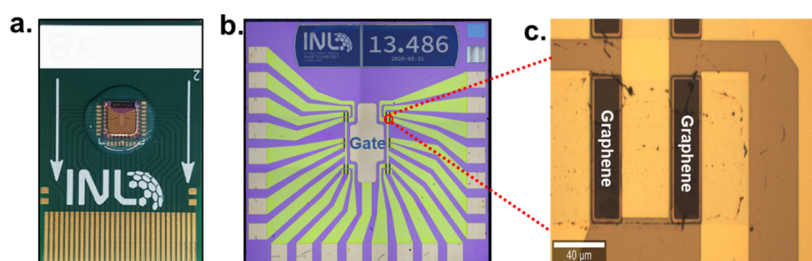
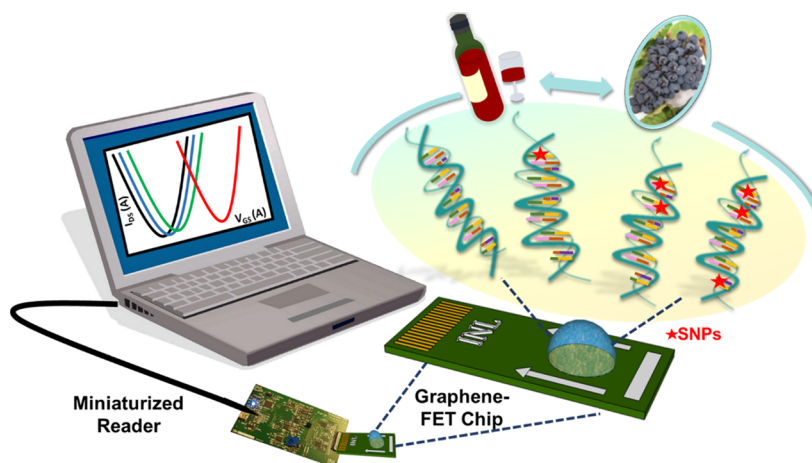


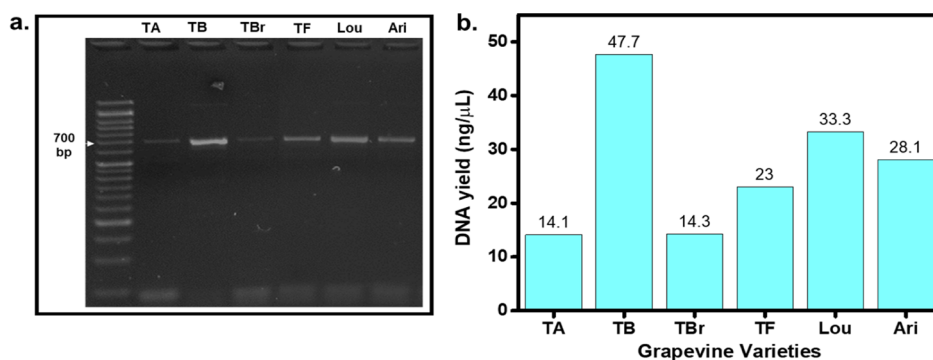
Figure 2. (a) Photograph of a ready-to-use GFET chip, (b) GFET chip layout, and (c) optical micrograph of the graphene channel on the chip.

pDNA and targets, namely, full complementary (FC), 1 mismatch (1 SNP), 2 mismatches (2 SNPs), 3 mismatches (3 SNPs), and NC sequences, were synthesized by Metabion International AG, Planegg, Germany (Table 1, with the bold letters referring to the SNPs' position). All the grapevine and wine samples corresponding to the SNPs' locations were from PDO Douro, including Tinta Amarela (TA), Tinta Barroca (TB), Touriga Brasileira (TBr), Tinta Francisca (TF), and provided by Sogrape (Portugal). All the sequence alignment was verified using the Geneious Prime 2020 software (<https://www.geneious.com>), as listed in Table S1. The other nontargeted (NT) varieties from Vinhos Verdes PDO, namely, Loureiro (Lou) and Arinto (Ari), respectively, were kind contribution of one of the authors. For the DNA hybridization study in wine matrices, we used the extracts from the wine containing the mixture of Ari and Alvarinho (Alv) varieties provided by Sogrape (Portugal). For DNA extraction of the grape berries, we used DNeasy PowerSoil Pro DNA from Qiagen (Hilden, Germany) and NucleoSpin Plant II from Macherey-Nagel (Düren, Germany). NucleoSpin Food from Macherey-Nagel (Düren, Germany) was used for wine extraction. The photograph of all the grapevine samples used in this study is shown in Figure S1.

**GFET Chip Fabrication and Electrical Characterization.** The general workflow for DNA detection using the GFET chip in this study is shown in Scheme 1. A detailed fabrication process for the GFET chip has been reported in our previous work.<sup>45</sup> The photograph of the ready-to-use chip is displayed in Figure 2a. In

brief, the chip was fabricated on a 200 mm Si oxidized wafer with 100 nm of thermal oxide onto which a 3 nm Cr adhesion layer, followed by a 35 nm Au layer and a 10 nm Al<sub>2</sub>O<sub>3</sub> sacrificial layer, was sputtered. The contact patterning was done into 513 square dies of 6.75 mm side by optical lithography and ion milling for etching the unprotected Cr/Au/Al<sub>2</sub>O<sub>3</sub> into the desired pattern. The GFET channel was fixed at 25 μm between source and drain contacts and a width of 75 μm. The chip layout is for 20 GFETs per chip, with one common in-plane recessed gate electrode, a common source electrode for each group of 10 FETs, and a back-gate access pad to allow double-gating of the transistors (Figure 2b). The single-layer graphene (SLG) growth was accomplished through CVD in a load-locked quartz tube 3-zone furnace (FirstNano EasyTube 3000) onto a high-purity copper foil. The graphene was grown on the catalyst copper foil from the gaseous mixture of methane/hydrogen at a gas flow rate ratio of 1:60 and a temperature of 1020 °C. The process was followed by graphene transfer onto the wafer, using a temporary polymeric substrate (polymethylmethacrylate, PMMA) wafer dicing into individual chips and packaging on a PCB. The graphene FET channel quality was checked by Raman spectroscopy using a WITec Alpha 300R system (WITec, Oxford Instruments Group) with a Zeiss microscope set of lenses. The 532 and 633 nm laser excitations in a backscattering geometry at a power output of 1.5 mW, a 50× lens objective, a numerical aperture of 0.7, and a 600 groove/mm grating for three 10 s acquisitions were set to collect Raman spectra at different sites of the





**Figure 3.** (a) Gel electrophoresis results, under optimal conditions, from DNA amplification with the conventional PCR from all the grapevine varieties used in this study, and (b) DNA concentration of the amplified products from all the grapevine varieties, ready for GFET sensor measurement.

graphene channel (depicted in an optical microscopy image shown in Figure 2c).

Graphene transfer curves were acquired after all the surface functionalization stages and DNA detection steps by inserting the chip into a card-size Arduino-compatible custom board consisting of a microcontroller, digital-to-analog and analog-to-digital (DAC, ADC) converters, a resistance-controlled current source of 1–100  $\mu$ A, a digital potentiometer, and complementary metal–oxide–semiconductor (CMOS) matrices. A constant source voltage ( $V_{DS}$ ) was applied at 1 mV. The electrolyte gating was performed by sweeping the gate voltage ( $V_{GS}$ ) from  $-1.0$  to  $1.0$  V with a voltage step of 15 mV. The device under test was put to the ground between steps. In this study, Dirac points, that is, the lowest point in the transfer curve, were extracted from arrays of up to 20 transistors per chip (malfunctioning transistors were excluded). For each measurement, three loops of data acquisition with 10 transfer curves per loop were collected from all the transistors to obtain a stable signal. Only the last loop was considered for the signal acquisition. The represented data are, therefore, an average of up to 20 graphene transistor signals successively read with a 1 s waiting time between transfer curves.

**Surface Functionalization and DNA Hybridization on the GFET Chip.** The surface functionalization stages have been reported in detail elsewhere.<sup>46</sup> First, the Au gate area was passivated with 2 mM DDT in ethanol to prevent the attachment of DNA and other organic molecules onto the Au layer. The graphene channel was then functionalized with 10  $\mu$ L of 10 mM PBSE in DMF for 2 h. The PBSE's ester group on its end tail enables the binding of the amine-tagged pDNA. The process is followed by the dropwise addition of 10  $\mu$ L of 10  $\mu$ M pDNA onto the GFET chip and incubation at 4  $^{\circ}$ C overnight in a humid chamber, after which the sensor is specific to the complementary tDNA strand. A blocking step with ETA is performed to minimize nonspecific binding. Finally, a series of tDNA sequences representing FC, 1 SNP, 2 mismatches (2 SNPs), 3 mismatches (3 SNPs), and NC groups were added and incubated for 1 h to allow the hybridization process. The tDNA was diluted with a hybridization buffer (HB) (0.01 $\times$  PBS/150 mM NaCl/50 mM MgCl<sub>2</sub>). The hybridization measurement started with a 1 aM tDNA concentration, followed by increasing concentrations in logarithmic steps until saturation was reached. 0.01 $\times$  PBS was used in all washing steps in between each surface functionalization step and signal collection.

**Validation of DNA Hybridization on a Chip Using a Confocal Microscope.** SLG on a copper surface was transferred onto a glass coverslip substrate. 10  $\mu$ L of 10  $\mu$ M pDNA was added dropwise onto this substrate functionalized with PBSE and incubated overnight to ensure probe immobilization, following the above-mentioned protocol. The substrate was then dried, and the Qubit ssDNA assay dye from the kit (Thermo Fischer Scientific, USA) was added dropwise onto the substrate and stood for  $\sim$ 5 min, washed, and dried. The substrate was then subjected to a confocal microscope (LSM780, Zeiss) observation under a 488 nm argon laser, channel-mode imaging setup, using a 63 $\times$  oil immersion lens. To investigate the hybridization of double-stranded DNA, we applied the same

protocol as above, using 10  $\mu$ L of 1  $\mu$ M tDNA for FC, 1 SNP, 2 SNPs, 3 SNPs, and NC, labeled with the Qubit dsDNA HS fluorophore (Thermo Fischer Scientific, USA) for  $\sim$ 5 min. The fluorescence dye is activated upon binding to the double-stranded DNA.

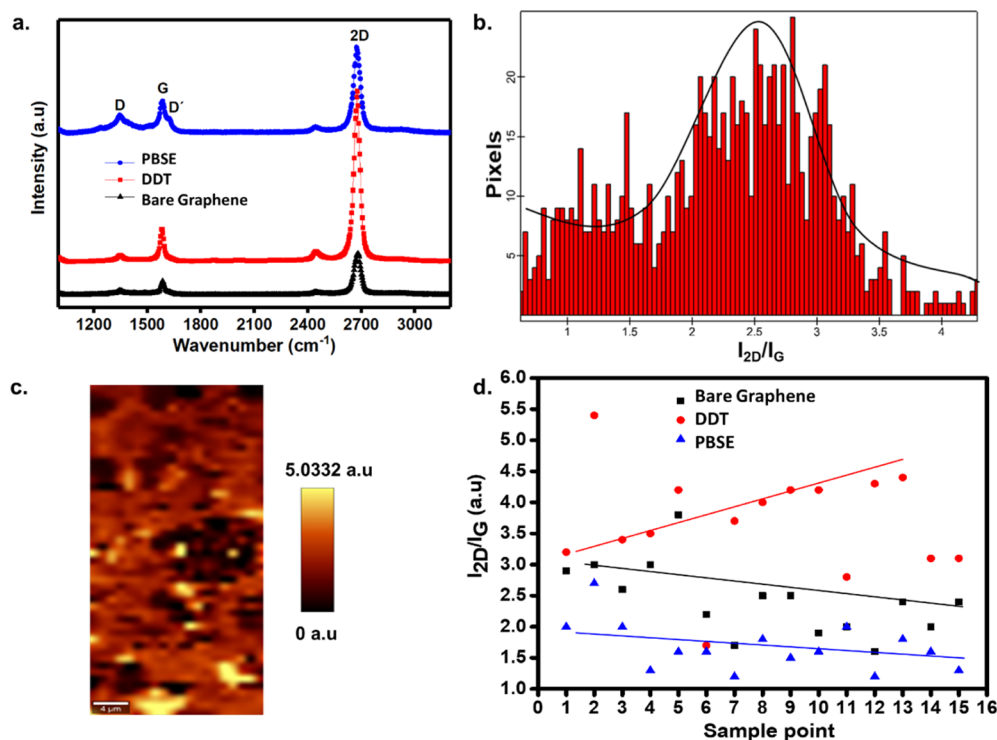
**DNA Detection from Grapevine Samples, Spike Analysis, and Wine Samples Using the GFET Chip.** The tDNA used in the protocol above was prepared from the DNA extracted from six grapevine samples, namely, TA, TB, TBr, TF, Lou, and Ari. The extraction was initially performed with two commercial kits, DNeasy PowerSoil Pro (PS) and NucleoSpin Plant II DNA (NSP). In this stage, we successfully optimized the DNA yield and purity based on the absorbance ratio value (Abs 260/280) with NucleoSpin Plant II DNA using SDS-based lysis (PL2) buffer (Figure S2). The amplification follows a PCR procedure reported by Gomes et al., 2018.<sup>9</sup> Detailed values of the investigated parameters for the qPCR temperature optimization based on melting analysis are listed in Table S2. The results are confirmed by gel electrophoresis (Figure S3). The outcomes of extraction and amplification optimization using PL2 buffer are supported by the melting peak data (Figure S4). We also verified the optimized results from the chosen extraction kit (NSP) using PL2 buffer by comparing it with the extracts from the PS kit in gel electrophoresis presented in Figure S5. Next, the total DNA from the amplified products was quantified using a Qubit fluorometer (Thermo Fisher Scientific, USA) before being used in the experiment with the GFET sensor.

The specificity analysis was performed by spiking the synthetic tDNA samples, fully complementary to pDNA (FC DNA, see Table 1), in a HB containing DNA extracts from NT grapevine varieties, namely, Lou and Ari. The FC DNA was also spiked in a HB containing DNA extracts from wine (a mixture of Ari and Alv), extracted with a NucleoSpin Food kit from Macherey-Nagel (Düren, Germany), following the manufacturer's instructions. The graphene FET sensor was ultimately used to detect DNA from wine samples. The DNA extraction from wine samples was performed under the NucleoSpin Food kit (Macherey-Nagel, Düren, Germany). Please refer to the Supporting Information and Figure S6 for more information on wine samples, DNA yield, and absorbance characteristics.

**Statistical Analysis.** For the statistical analysis results, all the standard deviations in the calibration plots were taken from triplicate measurements on each transistor in a chip under an identical experimental protocol. The dose–response fitting function from Origin 9 software (OriginLab, Northampton, MA, USA) was applied to perform the nonlinear regression of the collected data using eq 1 below

$$y = \frac{V_{\min} + (V_{\max} - V_{\min})}{1 + \left(\frac{10^{\log EC_{50}}}{10^x}\right)^p} \quad (1)$$

where  $V_{\min}$  is the minimum DNA hybridization rate,  $V_{\max}$  is the maximum DNA hybridization rate,  $EC_{50}$  refers to the concentration of



**Figure 4.** (a) Raman spectra of graphene as a function of surface functionalization, (b) histogram fitted by Gaussian curve, (c) two-dimensional  $I_{2D}/I_G$  map of the bare graphene channel, and (d) comparison of  $I_{2D}/I_G$  of graphene resulting from different surface functionalization steps (the lines are mere guides to the eye).

pDNA that produces a half-maximal tDNA hybridization, and  $p$  is the Hill slope. To estimate the limit of detection (LOD) of the GFET sensor, we calculated two initial parameters<sup>47</sup> using eqs 2 and 3 as follows

$$\text{Limit of Blank (LoB)} = \text{mean}_{\text{blank}} \pm 1.645 (\text{SD}_{\text{blank}}) \quad (2)$$

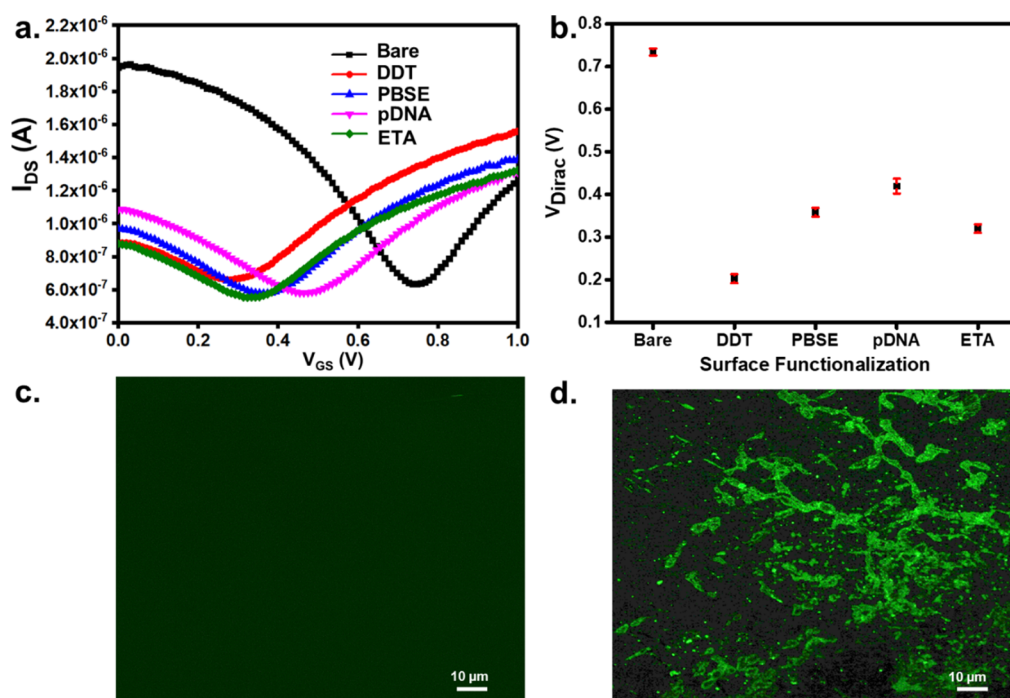
$$\text{LOD}_{\Delta V} = \text{LoB} \pm 1.645 (\text{SD}_{\text{lowest concentration}}) \quad (3)$$

## RESULTS AND DISCUSSION

In this study, we successively tested the synthetic tDNA's hybridization, extracted and amplified DNA from various grapevine varieties, spiked complementary tDNA in a HB containing complex matrices from NT grapevine varieties and a mixture of wine, and finally, the wine DNA samples. The initial part of this study focuses on optimizing the DNA extraction and amplification from grapevine to enrich DNA yield for detection in the GFET chip. Grapevine samples underwent sample pretreatment, namely, DNA extraction and amplification, and were then applied as complex matrix samples. The extraction optimization was obtained using a particular lysis buffer, and amplification protocols are explained in detail in the [Supporting Information](#). Figure 3 presents the optimization results in these pretreatment processes, including DNA extraction and PCR amplification. As seen in the figure, the amplified DNA from all grapevine varieties used in this study was confirmed by gel electrophoresis and quantified. It is demonstrated that the targeted band ( $\sim 786$  bp) from all varieties was present (Figure 3a). Our calculation (Figure 3b) shows that these DNA concentrations were approximately equal to  $\sim 100$   $\mu\text{M}$  synthetic tDNA. The amplified products were diluted with the HB and denatured at  $95^\circ\text{C}$  before the hybridization process with pDNA on the GFET chip.

Raman spectroscopy was utilized to ensure the quality of graphene after the transfer process and monitor the step-by-step surface functionalization. In Figure 4a, Raman spectra acquired before the surface functionalization stage confirm the properties of monolayer graphene presented by the 2D peak at  $\sim 2681$   $\text{cm}^{-1}$  and G peak at  $\sim 1585$   $\text{cm}^{-1}$  with an intensity ratio ( $I_{2D}/I_G$ ) of  $\sim 2.5$ , with a minor D peak ( $\sim 1347$   $\text{cm}^{-1}$ ). The quality of the transfer process is also verified by the absence of the PMMA peak ( $\sim 2900$   $\text{cm}^{-1}$ ). The homogeneity of the monolayer graphene is confirmed in the  $I_{2D}/I_G$  histogram (Figure 4b) taken from a 2D Raman intensity mapping of a  $20 \times 50$   $\mu\text{m}^2$  scanned area (Figure 4c). In Figure 4a, after the passivation of the Au surfaces with DDT, the significant peaks confirming the monolayer graphene (2D and G peaks) are not altered, implying that the graphene structure is not perturbed by the self-assembled monolayer formation on the Au contacts. Subsequently, in Figure 4a, after PBSE treatment, the 2D peak remains a single Lorentzian peak, and the ratio of  $I_{2D}/I_G > 1$  still reflects the monolayer character of graphene, which indicates that PBSE attachment onto graphene through  $\pi$ - $\pi$  interaction does not change its structure. However, we observed the D peak's distinct appearance, typically associated with the resonance of the pyrene group onto the graphene surface and an increased edge density. A new peak denoted as D' at  $\sim 1628$   $\text{cm}^{-1}$  appearing as a G peak's shoulder indicates the pyrene group vibrational mode, validating the signature of the noncovalently attached PBSE on the graphene surface. D and D' peaks resulting from a pyrene-based linker on graphene are consistent with the results reported by Nguyen et al., 2021.<sup>48</sup>

We also noticed that the full width at half-maximum of the 2D peak substantially broadened from  $\sim 39.96$   $\text{cm}^{-1}$  for bare graphene to  $\sim 45.08$   $\text{cm}^{-1}$  after PBSE immobilization, which



**Figure 5.** (a) Transfer curve characteristics of GFET from different stages of surface functionalization, (b) position of  $V_{Dirac}$  after each functionalization stage, and fluorescence micrograph of GFET substrate (c) before and (d) after pDNA immobilization.

could be the effect of a partially formed bilayer graphene/pyrene.<sup>49</sup> Raman measurements taken from five points (Figure S7) show the homogeneity of the PBSE functionalization. Figure 4d demonstrates that compared to the bare graphene  $I_{2D}/I_G$  ( $\sim 2.5$ ), the DDT treatment results in the increment of  $I_{2D}/I_G$  ( $\sim 3.5$ – $4.5$ ), while PBSE generates lower  $I_{2D}/I_G$  (1.3–2). Following studies by de Almeida et al., 2020,<sup>50</sup> and Kwong Hong Tsang et al., 2019,<sup>42</sup> the lower  $I_{2D}/I_G$  after PBSE is ascribed to graphene-increased structural disorder affirmed by the D peak existence.

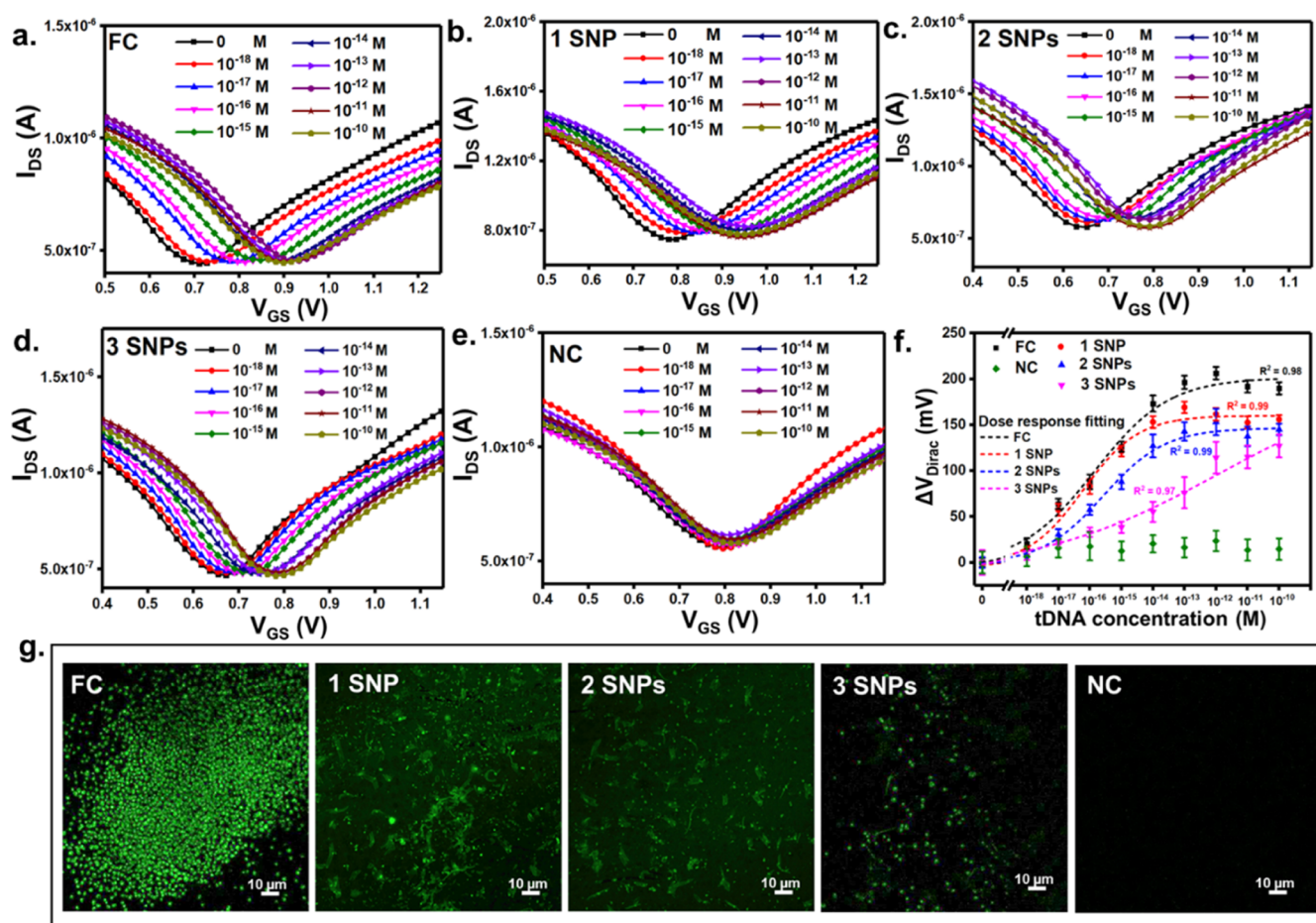
The functionalization steps were also monitored by the evolution of the liquid-gate transistor characteristics using the portable system. The liquid-gating mechanism modulates the graphene channel's conductance by accumulating counterions at the graphene interface, leading to graphene's p- or n-doping effects.<sup>51</sup> The ambipolar transport properties of graphene are reflected in the two branches of the transfer curve separated by the Dirac point at  $V_{Dirac}$ , which shifts depending on the graphene channel doping level. Figure 5a,b records the transfer curves and  $V_{Dirac}$  shifted values after each functionalization step. For the bare graphene channel,  $V_{Dirac} = 0.733$  V, indicating an initial, nonintentional p-doping, which is common in GFET cleanroom-processed devices.<sup>52</sup> After the SAM formation on the Au contacts, a one-time shift of  $V_{Dirac}$  ( $V_{Dirac} = 0.202$  V) is uncorrelated with graphene doping effects but results from the thiol–gold complex formed at the contact/electrolyte interface.<sup>53</sup>

The functionalization with the PBSE linker shifts  $V_{Dirac}$  to a more positive gate voltage concerning the DDT treatment ( $V_{Dirac} = 0.358$  V). This behavior aligns with our previous findings reporting that the  $\pi$ – $\pi$  stacking interaction of PBSE and graphene surface enhances the p-doping effects on graphene.<sup>52</sup> In the next stage involving the attachment of pDNA, the  $V_{Dirac}$  is moved toward a more positive voltage relative to PBSE, reaching the value of  $V_{Dirac} = 0.419$  V. This p-doping effect on graphene is a consequence of the

accumulation of the negatively charged phosphate oligonucleotides in the DNA backbone at the solid–liquid interface.<sup>52</sup> To minimize the nonspecific binding to the unreacted linker molecules and enhance the efficiency for specific DNA target hybridization, we treated the unoccupied linker with ETA blocking. The blocking stage successfully removes weakly attached pDNA to the PBSE linker, thus eliminating negative charges on graphene and shifting  $V_{Dirac}$  to a lower value ( $V_{Dirac} = 0.319$  V) concerning the pDNA signal. We also confirmed the attachment of pDNA onto the GFET channel using fluorescence signals from a confocal microscope using a highly specific dye from the Qubit ssDNA assay kit that would be activated in the presence of single-stranded DNA. Figure 5c,d depicts a clear difference in the fluorescence signal before and after the immobilization of pDNA onto the functionalized graphene substrate. It is observed that the drop-cast method for pDNA immobilization has excellently covered the surface with a rather homogeneous distribution of the probe molecules and validated the efficiency of the PBSE linker.

The electrostatics of the solid–liquid interface phenomena essentially screens out the charge accumulation within a volume set by the width of the Debye layer. In this study, 0.01 $\times$  PBS provides the Debye length ( $\lambda$ ) of  $\sim 7.61$  nm. It is calculated that the length of the PBSE linker plus the 35-mer pDNA is  $\sim 13$  nm. We hypothesize that all the hybridization occurs inside the electrical double-layer volume, one Debye length thick, considering that the DNA conformation is not fully upright or oriented perpendicular to the solid surface plane. Research on DNA immobilization on Au surfaces denoted that 21–50 bp dsDNA tilt at 40–50°, while another work using a CVD diamond substrate reports 36-mer ssDNA tilts at 50° and 29 bp dsDNA tilts at 52°. If we apply a similar analysis, the tilt angle  $\sim 40$ – $50^\circ$  (35 bp) can be equated to  $\arcsin(T/L)$  with  $T$  the DNA layer thickness and  $L$  the DNA length. In that case,  $L$  is  $\sim 8.7$  nm, indicating that >90% of the target sequence is within the Debye area, ensuring optimum



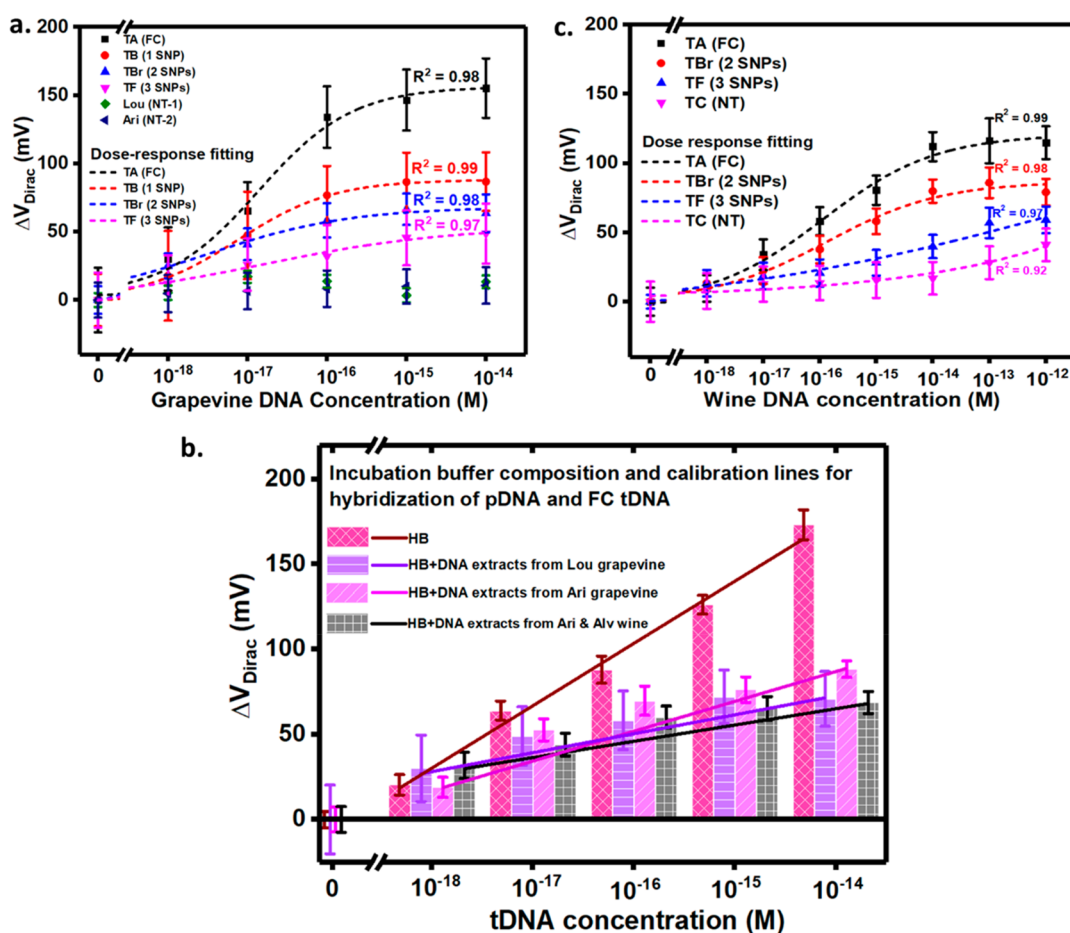


**Figure 6.** Transfer curve characteristics of GFET resulting from the DNA hybridization of (a) FC, (b) 1 SNP, (c) 2 SNPs, and (d) 3 SNPs. (e) NC sequences and (f) calibration plots of each hybridized DNA detected representing  $\Delta V_{Dirac}$  as a function of DNA concentration with dose–response fitting and (g) fluorescence micrograph of hybridized DNA with different mismatch degrees onto the GFET substrate.

signal acquisition during the whole measurement. The performance of the GFET chip is initially demonstrated in synthetic DNA hybridization detection with different degrees of SNPs. Each measurement was repeated three times for each group of hybridization tests, and the transfer curve data corresponding to the 10th transfer curve acquired in each series was used to plot the data. Each measurement was repeated on up to 20 different transistors. Hence, the results are an average of up to 20 independent measurements. As seen in Figure 6a–e, the transfer curves of GFET from the hybridization of FC, 1 SNP, 2 SNPs, 3 SNPs, and NC targets with pDNA produce a distinct  $V_{Dirac}$  shift with attenuated sensitivity as the number of mismatches increases. We define sensitivity as the change in  $V_{Dirac}$  per decade of the target concentration. Again, the  $V_{Dirac}$  shifting to higher gate voltages for higher tDNA concentration is a consequence of negative charge accumulation near the GFET surface upon double-stranded formation due to one negative charge added per base in the hybridized strand. The transfer curve records visually indicate that the higher degree of DNA mismatches results in weaker shifts of Dirac points. The calibration plot in Figure 6f shows analyte discrimination corresponding to the degree of DNA mismatches. The error bars are the standard deviation of triplicate measurements made on three chips produced and treated under the same conditions. The hybridization detection from FC, 1 SNP, 2 SNPs, and 3 SNPs was statistically significant under dose–response fitting, as shown by their  $R^2$

values. It is noteworthy that the saturation point comes later with more DNA mismatch, which explains the extended period required by the DNA with higher mismatches to match and fit their partially complementary nucleobases and finally immobilize at the graphene interface. Moreover, with the higher degree of mismatches, some weakly bound DNAs are easily removed during the washing steps, which reduces the  $\Delta V_{Dirac}$  throughout the measurement.

As the sample is a complex matrix of food, DNA extraction and purification from this complex matrix and target enrichment through DNA amplification is part of the standard protocol (Figure 1). The presented calibration curves for the specific tDNA sequence show a linear range and an estimated sensitivity representing how the calibration curves behave differently with different DNA mismatch levels in the samples, providing a different curve. In actual sample handling, DNA amplification when the sample concentration is too low and serial dilution when the sample concentration is too high concerning the GFET sensor's dynamic range would be critical before running a series of concentrations from unknown SNP numbers in the sample. The optimization and exploration toward simultaneous screening that reflects the efficiency of the process could be targeted in the future using a multiplex sensing layout. As for many other DNA hybridization-based sensors and as shown in Figure 6f, closely related DNA sequences, for example, those from very phylogenetically close grape varieties at high concentrations, will give a signal on our



**Figure 7.** (a) Calibration plots of each DNA hybridization measurement using grapevine samples with different SNP properties representing  $\Delta V_{\text{Dirac}}$  as a function of DNA concentration with the dose–response fitting, (b) performance of the GFET sensor tested for the hybridization of pDNA with FC DNA in HB and FC DNA spiked in HB containing DNA extracts from NT grapevines (Lou and Ari) and wine (mixture of Ari and Alv), and (c) calibration plots of DNA hybridization using wine DNA samples with different SNP properties representing  $\Delta V_{\text{Dirac}}$  as a function of DNA concentration with the dose–response fitting.

sensor. Therefore, there may be cross-reactivity for related varieties. However, we have demonstrated that, in case of doubt with blind monovarietal samples, the ambiguity is removed by analyzing two different concentrations, unequivocally discriminating between SNPs. We performed the linear fitting on each SNP detection and calculated the slope/sensitivity in the linear range region as our standard calibration curve. The signal from each SNP should fall within their standard calibration curve.

In 1 and 2 SNP hybridization, the calibration plot does not dramatically change the trend nor reduce the sensitivity, yet the signal distinction is clear. The similar sensitivity for the FC and single SNP might be linked with the mismatch position(s) at the edge of the sequence (close to the graphene interface) that leaves a significant portion of remaining complementary bases hybridized and affect the whole duplex stability. In contrast, a single SNP study in the literature<sup>55</sup> reported an extreme FET signal distinction. Our group also observed significant signal attenuation in previous work.<sup>52</sup> However, these studies utilize the SNP's position precisely in the center of the tDNA sequence. The influence of different mismatch positions is also demonstrated by Cheung et al. by placing a single base-pair mismatch at various fifth positions on 15-mer oligonucleotides.<sup>56</sup> Our outcomes reveal the high sensitivity and specificity of the SNP detection even with the SNP's

location far from the target sequence center. The NC target hybridization resulted in minimal conductance change on the graphene interface, leading to low sensor responses.

To confirm and support the electronic sensor detection of the DNA hybridization, we perform a fluorescence imaging of each FC, 1 SNP, 2 SNPs, 3 SNPs, and NC hybridization onto functionalized graphene substrates using the CLARIOStar Qubit dye that promptly intercalates the double-stranded DNA. The surface fluorescence image, therefore, reveals the areal distribution of hybridized strands. Figure 6g shows a decrease in the fluorescence signal from the FC, 1 SNP, 2 SNPs, 3 SNPs, and NC hybridization on graphene. The FC hybridization achieved a dense and even fluorescence coverage. The fluorescence signal after probe hybridization gradually weakens as the target contains higher SNPs. Figure 6g also shows that the NC results did not generate any fluorescence signal, indicating low or nonoccurring hybridization.

The GFET portable sensor was further employed to detect the grapevine varieties based on specific DNA sequences. As shown in the calibration plot in Figure 7a, the hybridization of targets from amplified DNA of the TA variety, with an entirely complementary sequence against the pDNA, yielded the highest response ( $\Delta V_{\text{Dirac}}$  over the target concentration). A lower  $\Delta V_{\text{Dirac}}$  value with gradual signal decrement is exhibited by TB, TBr, and TF, representing the 1 SNP, 2 SNPs, and 3

SNPs in the selected DNA sequences, respectively. The transfer curves of the DNA hybridization measurement from the amplified grapevine sample are shown in Figure S8. The dose–response fitting has given the best fit with  $R^2 > 0.95$  for all the grapevine DNA hybridization from varieties with complementary and SNP targets. Compared to the hybridization data from synthetic DNA, higher standard deviations are displayed in the hybridization test using grapevine DNA caused by the complexity of the longer DNA strands in the grapevine samples. In contrast, being tested with the DNA extract from NT grapevine varieties, namely, Lou and Ari, negligible shifting trends of  $V_{\text{Dirac}}$  over NT DNA concentration were recorded. The overall outcomes show the high specificity of the pDNA immobilized on graphene applied in this study. In Figure 7b, we compare the calibration plots for DNA hybridization using FC tDNA (FC DNA) (see Table 1), which is the synthetic oligonucleotide complementary to the pDNA, and using an FC DNA spiked in a HB with DNA extracts from NT grapevines (Lou and Ari) and wine (mixture of Ari and Alv). The HB with and without NT grapevine's DNA extracts was tested for the hybridization step. After washing and measuring with  $0.01\times$  PBS, the obtained sensitivity was 12 and 22 mV/decade for hybridization of pDNA with FC DNA spiked in HB containing DNA extracts from Lou and Ari grapevine varieties, respectively. In the case of FC DNA spiked in a HB containing extracted DNA of the wine mixture, the sensor's sensitivity was  $\sim 10$  mV/decade. As expected, the spiked analysis yielded lower sensitivity than FC DNA detection after incubation in a pure HB. Nevertheless, the positive shifting trend in  $\Delta V_{\text{Dirac}}$  was maintained, ascribed to the high specificity of the proposed GFET sensor even when tested using the grapevine and wine matrices. The complete transfer curves and fittings are presented in Figure S9.

To test the applicability of the GFET sensor toward wine detection, we carried out the hybridization signal measurement with DNA from monovarietal wine samples, including the TA (FC), TBr (2 SNPs), TF (3 SNPs), and Tinto Cão (TC) NT (Figure 7c). It is shown that the calibration trends for wine samples are similar to those obtained in synthetic and grapevine DNAs, where signal attenuation takes place with the higher SNPs contained in the sample. Most plots provide a highly confident data set for dose–response fitting, reaching  $R^2 > 0.95$ . Observing the calibration plots, the lower sensitivity in the wine sample detection compared to grapevine is predictable due to the more complex matrices of the original sample. However, the signal differentiation by different levels of DNA mismatches in monovarietal wines straightforwardly discriminates the grapevine variety as the raw material used throughout the winemaking process. It also signifies the suitability of the fabricated portable sensor for rapid detection in varietal discrimination, down to low DNA mismatch numbers, which eliminates the need for time-consuming and pricey sequencing analysis or other DNA-based analysis requiring costly and sophisticated equipment.

In a statistical analysis, we performed two fittings, the nonlinear regression using dose–response fitting to proceed with the LOD calculation and the linear fitting to screen the sensitivity of the GFET sensor in the detection of samples with different degrees of mismatches, both using the synthetic DNA and grapevine samples. According to the dose–response fitting outcomes,  $R^2$  in all groups has a value  $>95\%$ . Based on the curve fitting analysis, the  $EC_{50}$  value, that is, the DNA concentration that elicits a response halfway between the

baseline and maximum response, lies around  $10^{-16}$  to  $10^{-15}$  M in synthetic and grapevine groups. Regarding the detection limit, in synthetic DNA screening, the higher mismatch increases the detection limits, while the FC hybridization outstandingly yields the smallest LOD at 0.19 aM. It indicates that the output signal was not significantly interfered with by the background solution, even for the lowest concentration tested on the attomolar level, and that this exceedingly low target concentration showed the capability to hybridize with the pDNA on the GFET chip. In the grapevine samples, all the detection limits still fell in the attomolar range. However, the LOD did not show disparities for different SNPs that could be linked with the higher interference from the more complex matrices than in the synthetic DNA samples.

Sensitivity is pivotal in assessing the FET biosensor performance. The complete set of calibration plots with linear fittings for all the targets and conditions tested is provided in Figure S10. The main features in the calibration plots have been translated into a detailed list of sensing performance parameters in Table 2 below. In the hybridization screening

**Table 2. Analytical Parameters of DNA Detection on the GFET Sensor with Synthetic, Grapevine, and Wine DNA Samples**

| sample                      | dose–response fitting |          | linear fitting |                         |                      |
|-----------------------------|-----------------------|----------|----------------|-------------------------|----------------------|
|                             | $R^2$                 | LOD (aM) | $R^2$          | linear range (M)        | sensitivity (mV/dec) |
| synthetic DNA               |                       |          |                |                         |                      |
| FC                          | 0.98                  | 0.19     | 0.98           | $10^{-18}$ – $10^{-12}$ | 36                   |
| 1 SNP                       | 0.99                  | 1.16     | 0.99           | $10^{-18}$ – $10^{-13}$ | 32                   |
| 2 SNPs                      | 0.99                  | 3.39     | 0.99           | $10^{-18}$ – $10^{-12}$ | 26                   |
| 3 SNPs                      | 0.97                  | 524.23   | 0.95           | $10^{-18}$ – $10^{-13}$ | 11                   |
| NC                          | n.a                   | n.a      | n.a            | n.a                     | n.a                  |
| grapevine DNA               |                       |          |                |                         |                      |
| Tinta Amarela (FC)          | 0.98                  | 9.94     | 0.92           | $10^{-18}$ – $10^{-14}$ | 32                   |
| Tinta Barroca (1 SNP)       | 0.99                  | 18.55    | 0.91           | $10^{-18}$ – $10^{-15}$ | 22                   |
| Touriga Brasileira (2 SNPs) | 0.98                  | 2.14     | 0.98           | $10^{-18}$ – $10^{-15}$ | 14                   |
| Tinta Francisca (3 SNPs)    | 0.97                  | n.a      | 0.97           | $10^{-18}$ – $10^{-15}$ | 11                   |
| Loureiro (NT-1)             | n.a                   | n.a      | n.a            | n.a                     | n.a                  |
| Arinto (NT-2)               | n.a                   | n.a      | n.a            | n.a                     | n.a                  |
| wine DNA                    |                       |          |                |                         |                      |
| Tinta Amarela (FC)          | 0.98                  | 10.81    | 0.99           | $10^{-18}$ – $10^{-14}$ | 23                   |
| Touriga Brasileira (2 SNPs) | 0.97                  | 3.51     | 0.98           | $10^{-18}$ – $10^{-14}$ | 16                   |
| Tinta Francisca (3 SNPs)    | 0.90                  | 5.80     | 0.97           | $10^{-18}$ – $10^{-14}$ | 14                   |
| Tinto Cão (NT)              | n.a                   | n.a      | 0.92           | n.a                     | n.a                  |

using synthetic DNA samples, similar to what is observed with the LOD trend, the higher mismatches of DNA tend to produce a lower sensitivity signal. The FC hybridization yields the highest sensitivity with 36 mV/decade tDNA concentration. A gradual sensitivity attenuation is observed with the SNP number in the target samples. Our platform's robustness is also demonstrated by the sensitivity from hybridization detection using grapevine samples, where the FC variety TA achieved high sensitivity at a 32 mV/decade DNA concen-



tration. The reduction in sensitivity as a function of the mismatch degree in the grapevines' DNA samples was also well established.

On the contrary, using grapevine samples, paralleling the tendency in the sensitivity, TA (FC variety) achieved the broadest dynamic range and reached saturation at  $10^{-14}$  M, while other samples with mismatches reached saturation earlier at  $10^{-15}$  M tDNA concentration. These observations suggest a higher hybridization competition rate in the more complex matrices provided by grapevine samples. The negligible signal yielded by the NC hybridization in synthetic and grapevine samples shows the highly sensitive and specific sensing capability. TA (FC) wine variety achieves the highest sensitivity in wine samples. The trend in sensitivity attenuation was recorded as the SNP number in the wine samples increased, as represented by TBr (2 SNPs) and TF (3 SNPs), respectively. Unsurprisingly, we observed that the dynamic range in the wine sample is not much altered from that obtained from the grapevine samples, which implies the high specificity of the pDNA and graphene FET sensor for detecting complex matrices not only in unprocessed food but the processed ones, like wine samples. The negligible signal from the hybridization of the pDNA and targets from the wine with NT variety also supports the system's accuracy.

Currently, there is no standardized protocol for varietal differentiation by DNA-based analysis to be deployed to the wine industry, and sophisticated analyses, such as those based on DNA sequencing, require the sample to be sent to specialized laboratories with highly trained staff and costly equipment. Our presented proof-of-concept system paves a significant pathway toward enabling decentralized analysis. At this stage, our study targets highly valued monovarietal wines. This paper does not deal with samples containing complex SNP mixtures. However, we show that the sensor provides individual calibration curves, one per SNP, in addition to the FC calibration curve. The slope/sensitivity in the linear range of each SNP has been calculated and is listed in Table 2 for reference. The most straightforward approach to estimate wine mixtures with more than one variety might be achieved by immobilizing a sequence from one particular variety on several GFET chips. The complementary target should show a high  $\Delta V_{\text{Dirac}}$  signal and be used as the reference. When samples are tested, if there is a significant  $\Delta V_{\text{Dirac}}$  signal at least at a nearly similar range as the reference signal, it suggests that the sample primarily contains the referenced variety. If a shift is not as significant as the reference signal, the sample may be a mixture of the referenced variety and others. If there is no signal, it indicates that the sample does not contain any referenced variety. Table S3 summarizes the applicability mechanism of our system. In future platforms, these curves can be loaded to a sensor memory and used to calculate the actual concentrations in samples containing the FC and mixtures of several SNPs obtained by acquiring multiplexed measurements at different sample dilutions followed by statistical data treatment. More complex electronics need to be added to the readout electronics to store this data and perform statistical computations.

The performance factors' comparison with previously published work in DNA-sensing devices is compiled in Table 3, highlighting that our portable GFET DNA sensor performs at the highest level in all main sensing performance parameters, including its robustness and ultrasensitivity with attomolar detection limit, high specificity, wide dynamic range, and

Table 3. Performance Comparison of the Previously Published DNA Sensors and the Present work

| target                                                                                           | sensing platform                              | sensing material                             | amplification step                                          | dynamic range             | LOD           | refs      |
|--------------------------------------------------------------------------------------------------|-----------------------------------------------|----------------------------------------------|-------------------------------------------------------------|---------------------------|---------------|-----------|
| tDNA with a hairpin probe                                                                        | optical (fluorescence)                        | graphene oxide (GO)                          | isothermal circular strand-displacement polymerase reaction | 0.01–40 nM                | 4 pM          | 60        |
| human immunodeficiency virus (HIV) oligonucleotide sequence associated with the HMDNAzyme (PHIV) | optical (chemiluminescence)                   | GO–horseradish peroxidase-mimicking DNAzymes | DNAzymes                                                    | 0.1–3 nM                  | 34 pM         | 61        |
| synthetic DNA                                                                                    | FET                                           | plasma treated-CVD graphene                  | n.a                                                         | 10 aM–100 fM              | 10 aM         | 62        |
| mRNA in plasma                                                                                   | FET                                           | p-typeMOS–FET and microfluidic               | RT-qPCR                                                     | 1 aM–1 fM                 | 84 aM (dsDNA) | 63        |
| DNA fragments                                                                                    | multifrequency impedance                      | paramagnetic beads microfluidic              | PCR                                                         | 0.039 aM–7.8 fM           | 3.9 aM        | 64        |
| synthetic DNA                                                                                    | fluorescence resonance energy transfer (FRET) | graphene oxide                               | n.a                                                         | 1–7 $\mu$ M               | 0.12 $\mu$ M  | 65        |
| DNA mismatches                                                                                   | electrochemical                               | AuNP-reduced GO                              | horseradish peroxidase functionalized carbon sphere         | $10^{-17}$ – $10^{-13}$ M | 5 aM          | 66        |
| synthetic DNA                                                                                    | optical                                       | optical microfiber                           | n.a                                                         | 1 pM–1 nM                 | 75 pM         | 67        |
| P53 gene DNA                                                                                     | electrochemical                               | zirconia-reduced graphene oxide-thionine     | n.a                                                         | 0–500 nM                  | 24 fM         | 68        |
| DNA–RNA synthetic and in human serum                                                             | FET                                           | deformed graphene                            | n.a                                                         | 2 aM–2 $\mu$ M            | 0.6 aM        | 69        |
| grapevine and monovarietal wine DNA                                                              | FET                                           | monolayer graphene                           | PCR                                                         | 1 aM–0.1 nM               | 0.19 aM       | this work |

portability. This summary also implies the promising features of our GFET sensor toward more expansively modern diagnostics harnessing the SNPs, such as in cancer detection where the slightest amount of DNA mutation could lead to protein malfunctions and become the early onset of metastasis.<sup>57</sup> Alternatively, personalized medicine could be targeted through SNP detection to predict the patient's response to medical treatments and therapeutics, the drug's adverse effects, and resistance.<sup>58</sup> In the broader outlook, including environmental control, considering the ultralow concentration screening, the proposed sensor could be applied as an early detector of invasive species in an ecosystem.<sup>59</sup>

## CONCLUSIONS

We fabricated a miniaturized DNA-sensing chip based on graphene FET sensor arrays that operate in a simple and label-free protocol with ultrasensitive and highly specific detection. The charge-based measurement using our graphene FET chip is easily achieved using phi–phi-based biomolecular capture onto the interface that preserves graphene's unique electrical properties. The proposed chip with arrayed transistors provides the robustness of a label-free measurement and has a high potential for a multiplex sensing setup. Whereas developed for grapevine varietal discrimination in investigating monovarietal wine authenticity, the graphene FET sensor can also be used as an analytical tool in other ampelographic work. We have unraveled the potencies of the GFET sensors toward wine authenticity control using complex and highly processed samples represented by grapevines and wines, respectively. It yields analytical outcomes regarding varietal discrimination with superior sensitivity and specificity, represented by SNPs. To the best of our knowledge, this portable sensing platform is the first used to distinguish grapevine varieties that show potential for monovarietal wine authenticity control based on DNA hybridization analysis. It is discernible that the performance-limiting factor is not only the sensitivity but also the specificity, as demonstrated in DNA detection in complex matrices. Overall, the proposed platform enables high-performance biosensing modalities through its exceedingly low detection limit below the attomolar range, wide dynamic range, high sensitivity and specificity (down to single mismatch detection), rapidity, and portability. The system could be regarded as an alternative route for the time-consuming and high-cost process of wine authenticity identification via classical DNA-based analysis by PCR and sequencing. In our future study on wine authenticity, we aim to establish multiplex sensors and integrate DNA extraction, amplification, and detection modules in a single micrototal analysis system ( $\mu$ TAS) for on-site and decentralized testing in various control points. We are also interested in low-concentration DNA and using aptamers to detect other target molecules as human disease biomarkers to widen our research scope. On a broader framework, considering the applicability and high selectivity in detecting SNPs, the proposed GFET sensor is potentially applied for expansive applications, such as early disease diagnostics like cancer screening, to target reliable and quick discrimination of the mutant and wild-type DNA sequences. Another example of the GFET sensor's potential application is DNA screening to detect invasive species in specific ecosystems.

## ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acssensors.2c02090>.

Alignment of DNA sequences with the SNPs' position; optimization for grapevine DNA extraction; extraction with DNeasy PowerSoil Pro kit; extraction with NucleoSpin Plant II; DNA amplification by PCR and verification with gel electrophoresis; temperature optimization for qPCR; DNA extraction optimization verified with PCR; wine DNA extraction with NucleoSpin Food kit; reproducibility of PBSE-functionalized graphene by Raman spectroscopy; detection of the DNA from grapevine samples and spike test using the GFET biosensor; and varietal discrimination mechanism diagram (PDF)

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## Notes

The authors declare no competing financial interest.

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