



Label-free optical biosensor for direct complex DNA detection using *Vitis vinifera* L.



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ABSTRACT

The ability to detect and quantify small amounts of DNA in biological complex samples is a hot research area. Up until recently most of the work performed in this area used label-dependent protocols that increases its complexity and overall costs. The aim the work was to develop a label-free technology suitable for DNA detection and quantification using real complex DNA samples. The applicability of this system was tested using synthetic ssDNA targets that guaranteed the systems specificity, in the sense that only complementary sequences hybridized with the probe. When using real samples extracted from *Vitis vinifera* L. the system was able to successfully detect and quantify the DNA present without any of the time consuming and costly amplification steps. The detection and quantification limits of the proposed system were 60 ± 20 nM and 201 ± 20 nM, respectively for Target 1 concentrations between 31 and 350 nM. This method can easily be applied to other species and purposes, allowing the direct detection of DNA in a label-free environment with high accuracy and specificity.

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1. Introduction

Ever since the discovery of the DNA base-pair coupling structure, the number of publications where this complex biomolecule is used as a sensing device has grown exponentially. One area that has taken a major interest in the DNA base-pairing structure is the development of biosensors [1,2]. The use of DNA as a structural base for biosensing development has numerous advantages, such as: (1) the thermostability of the molecule when compared to other biomolecules, *i.e.* proteins; (2) highly conserved in the sense that replication has a very low mutation rate, allowing an accurate fingerprinting (3) the presence in all the cells, thereby potentially enabling identical information regardless of the tissue origin and the transformation process that they were submitted [3,4].

A DNA-based biosensor can be of the outmost importance in areas such as, food traceability and authentication, clinical diagnostics, gene therapy, biomedical studies, among others [5–8].

Over the past few years there has been some efforts in order to develop biotechnologies that will improve both sensitivity and

selectivity when considering gene analysis [8–10]. Usually these analysis require DNA labelling with a fluorophore. In some particular cases where signal amplification is the most critical issue, such as, ultratrace gene analysis, the DNA hybridization is followed using a fluorescent dye [1,11]. However fluorophores have numerous disadvantages that have reflection's in the sensitivity of the method, namely low photostability, photobleaching, low signal amplification, particularly in biological tissue where self-fluorescence is a major issue, among others [12]. Moreover, the DNA probe can only be labelled with one or a few fluorophores, which results in a weak signal, particularly when the target concentration is low. This limits not only affect the method's sensitivity but also the detection and quantification limits.

In order to overcome these issues, some work has been developed in terms of nanoparticles to support the DNA or a fluorescence label. In fact, and even though some interesting values have been reported [1,6,13,14] for the detection limit, these alternatives still require DNA labelling.

Other DNA-based technologies rely on amplification procedures, based on Polymerase Chain Reaction (PCR) previous to detection, such as real time PCR that additionally requires an amplification in order to detect the target and quantify it, with some success limitation when PCR-inhibitors are present in the sample

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Table 1
Sequences of the oligonucleotides used throughout this work:

Oligonucleotides	Sequence
ssDNA – Probe	5'-C6-Aminolink GGTGAAATGGGCACCGAACACACGC-3'
Target ₁ (Complementary)	5'-GCGTGTGTTCCGGTGCCCATTTACC-3'
Target ₂ (Non-Complementary)	5'-AAAAAAAAAAAAAAAAACCATTTACC-3'
Target ₃ (One Base Mismatch)	5'-GCATGTGTTCCGGTGCCCATTTACC-3'
Target ₄ (Six Bases Mismatch)	5'-GCATGTGTTTTTGGCCCATTTACC-3'
Target ₅ (Complementary with a tail of 24 bases in 5')	5'-TCTCTCTCTCTCTCAGCAAGGAAGCGTGTGTTCCGGTGCCCATTTACC-3'

[15]. Alternative to these methods is the use of fiber optic SPR for DNA hybridization detection [16] however, these options require expensive equipment and specialized personnel.

In this work a simple, low cost method for label-free DNA detection and quantification was developed, producing competitive results. This method is based on the ssDNA (DNA-probe) immobilization in the lateral surface of an optical fiber long period grating (LPG) and subsequent hybridization. The DNA is not dye-labelled and the hybridization is followed *in situ* by differences induced on the optical fiber surrounding media refractive index. More, the process does not require enzymatic reactions. This system was successfully applied for the detection of synthetic ssDNA targets (complementary, non-complementary and partially-complementary), as well as, genomic DNA previously extracted from *Vitis vinifera* L.

2. Material and methods

2.1. Materials

All oligonucleotides used in this work were purchased from Frilabo. The stock solutions were prepared with ultrapure water and stored at -20°C . Each solution contained $100\ \mu\text{M}$ of each oligonucleotides. The oligonucleotides sequences are presented in Table 1 and was based on the specific primer of *V. vinifera* designed to detect Single Sequence Repeats [17].

For each experiment a suitable amount of the stock solution was diluted in saline phosphate buffer (PBS: 10 mM sodium phosphate; 120 mM NaCl; 2.7 mM KCl; pH 7.4) in order to obtain the following concentrations: 0.50; 0.25; 0.125; 0.0625; 0.03125; 0.015625 and 0.007812 μM . All other chemicals were used without further dilutions.

The cleaning solution used before each experiment was composed by Ethanol 70% (v/v) and 1% Hydrochloric acid (v/v) in a (1:1) ratio. Additionally the restringing solution was a mixture of PBS with 0.1x Saline-Sodium Citrate (SSC) and 0.1% Sodium dodecyl sulphate (SDS) in a (1:1) ratio. After each cycle the LPG was cleaned using a diluted solution of Nitric Acid (HNO_3 1:3).

2.2. Instrumentation

The detection system is based in a fiber Long Period Grating (LPG) sensor. This sensor is an optical wavelength band-loss filter where the central wavelength of the band depends of the LPG fabrication parameters, temperature, applied axial mechanic tension and fiber surrounding media refractive index. It is also known that the double strand DNA has, in solution, a slightly higher refractive index than the single strand form. The detection principle of this particular LPG sensor is based on the use of a single DNA strand (Probe) attached to the fiber's lateral surface. When the probe hybridizes with its complementary strand, it becomes a double strand DNA increasing the fiber's surrounding refractive index media that, in turn, will have an effect on the LPG transmission spectra that is the data measured.

For that purpose the LPG sensor was placed inside of a wet flow cell that will provide the means to maintain a constant mechanic

tension applied to the fiber and, simultaneously allows the insertion and removal of wet solutions in the sensor surrounding zone which has been detailed in Gonçalves et al. [18]. As consequence, the wavelength variations accounted can only be due to the interaction of the solution with the LPG surface. The chamber capacity is of $750\ \mu\text{L}$ and the same volume of each solution was injected into the chamber in order to maintain the volume constant. The data acquisition was performed using a fiber optic interrogation unit manufactured by Fibersensing[®], model BraggMeter FS2200SA, with two channels modified to allow the measuring of the transmission spectra in the spectral region between 1500 and 1600 nm.

The sensor apparatus was maintained at constant temperature by inserting it in a muffle (Termarks, model B 8023). The sensor temperature was measured by a type-K thermocouple positioned in contact with the sensor chamber and its value were recorded by a temperature logger (Keithley[®] 740) controlled by the same computer that was measuring the spectra using a Labview[®] program creating a temperature list with a temporal tag for each temperature value. In this way, both spectra and temperature measurement where time synchronized and can be co-related.

Upon data acquisition the spectral data is processed to determine the position of the wavelength LPG resonance as described by Gonçalves et al. [18].

2.3. DNA extraction

In order to test the applicability of the sensing system into a more complex DNA matrix, a sample of the *Vitis vinifera* L. DNA was tested. The genomic DNA was extracted from leaf samples using the cetyl trimethylammonium bromide (CTAB) method described by Doyle and Doyle [19]. The extracted DNA was resuspended in $50\ \mu\text{L}$ of distilled water and the DNA concentration was determined using a NanoDrop[®] ND-1000 spectrophotometer. The DNA quality was assessed using a 0.8% (w/v) agarose gel stained in $7\ \mu\text{g mL}^{-1}$ ethidium bromide solution.

2.4. Chemical sandwich sensing system preparation

The LPGs were inserted in a glass chamber and the strain was fixed. Before performing any tests the LPG surface was cleaned by the passage of ethanol 70% (v/v) and hydrochloric acid 1% (v/v) solution in a 1:1 ratio.

The LPG surface is negatively charged, so is the DNA, as such, it was necessary to use a bilinker. In this work, the bilinker chosen was Poly-L-Lysine (PLL). In each cycle the following sequence was used: water, PLL, Probe, Targets or DNA from *V. vinifera*. All samples used were set at $0.25\ \mu\text{M}$. All measurements, with the exception of water added in between the addition of an analyte, were performed over 30 min, at 37°C . Upon the hybridization the target was removed from the LPG surface by a process called stripping. This was performed at 60°C and included the addition of a stripping solution (0.1x Saline-Sodium Citrate and 0.1% Sodium Dodecyl Sulphate in a (1:1) ratio), ethanol and water. In the end of each cycle all chemicals were removed from the LPG surface, using a diluted solution of HNO_3 (1:3, v/v) that was added and left in contact with the LPG for 15 min.

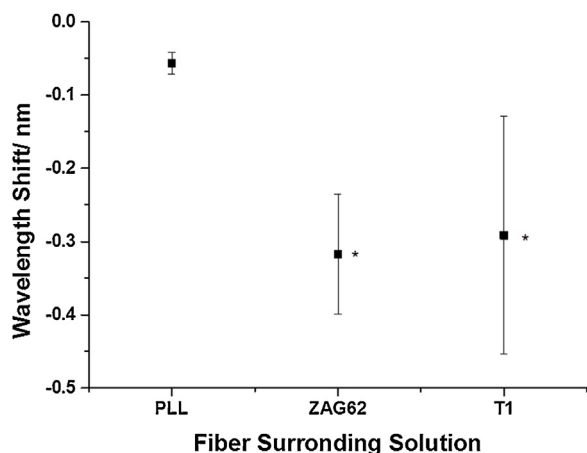


Fig. 1. Representation of the signal obtained using a simple ssDNA (Probe), an amino-terminated ssDNA (ZAG62) and Target 1. *The values are statistically significant when compared to the Probe Signal for a $p < 0.05$.

2.5. Chemical sandwich sensing system preparation

In order to evaluate the statistical significance of the data obtained throughout this work an IBM Software Package Statistical Analysis (SPSS) version 19 was used. The data were analysed in comparison to the Probe and the PLL signal, for the targets and the ZAG62 evaluation, respectively, and were considered statistical different when $p < 0.05$.

3. Results and discussion

3.1. The chemical interaction between the sensing system

A common problem in immobilized biomolecule methods is the need that even though the DNA-probe is covalently-attached to the fiber it should still interact with the targets and hybridize. This problem was surpassed by the use of a polymer—Poly-L-Lysine (PLL) [20]. This polymer has a positive charge which makes it suitable for a single sandwich system—fiber (negative charge); PLL (positive charge); DNA-probe (negative charge). Indeed, even though PLL is commonly used as a bilinker for DNA attachment, the chemical interactions between this system are not fully understood. In order to better understand the chemical relationship that lied beneath the sandwich sensing system an experiment was devised. According to authors, such as Zibai et al. [20], PLL forms a monolayer that links to the optical fiber surface through a hydrogen bridge bound between the Si-OH groups of the fiber and the PLL amino group. Additionally these authors suggest that the linking between DNA and PLL also occurs through an amino link. However what remains to be explained is if the absence of the amino-terminated groups in the DNA is determinant for the hybridization to occur. We wanted to test this hypothesis and used amino-ssDNA and DNA without an amino group. The results presented in Fig. 1, show that their statistical difference between the PLL signal and the amino free DNA (ZAG62), as such, the ZAG62 can effectively interact with the immobilized PLL even though it does not have an amino group. However, when comparing these signals with Target 1 it is possible to say that there is no statistical confirmation for hybridization. This behaviour can be due to an orientation effect that the amino group confers to the ssDNA. Indeed the interaction between the PLL and the ZAG62 or Probe, can be due to hydrogen bonding between the DNA residues and the polymer, but when there is an amino group in the ssDNA extremity (Probe), the interaction between PLL and the Probe is more significant due to the stability of the N–N chemical bonding. Additionally, when

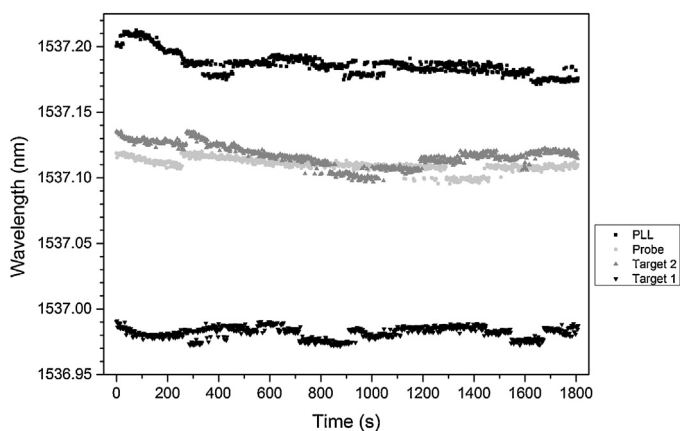


Fig. 2. Representation of the wavelength evolution with time registered for 30 min within each step PLL Deposition, Probe immobilization, Target 2 (non-complementary) and Target 1 (hybridization).

the interaction between the polymer and the DNA is due to this N–N bonding the position for this interaction favours the subsequent hybridization. On the other hand, when the ssDNA and the PLL is not specifically oriented this interaction can place the ssDNA (ZAG62) in such a way that the hybridization cannot be performed. This position is so variable that it is possible to see a hybridization in one cycle and not see it in the next, which can be responsible to the high standard deviation found for Target 1 in these particular experiments.

3.2. Stability and reproducibility of the sensing system

The stability of the sensor is an important issue. In order to define the time requested to obtain a stable signal a study concerning a time frame of 1800 s was undertaken using PLL, Probe, Target 2 and Target 1. The wavelength signal was recorded during 30 min (Fig. 2) and stabilization of the reaction on the fiber within each analyte was obtained after 20 min. All the further readings were done taking into consideration this time frame.

Another issue that needed to be addressed was the low reproducibility that is often reported when using fibers with long period gratings (LPGs), depending on the thickness of the interaction region and the penetration depth of the evanescent [21]. Indeed when using different LPGs it is necessary to perform a calibration that can be slow due to its singular nature. In order to overcome this problem a new strategy was developed, where the LPG was put in contact with a diluted solution of nitric acid. This allowed the use of the same LPG for more than 20 complete assays over more than 5 months.

In order to evaluate the method sensitivity and selectivity five different strands of DNA were tested. Target 1, Target 2, Target 3, Target 4 and Target 5 (Table 1). Target 1 is complementary to the DNA-probe, Target 2 is non-complementary, Target 3 has a single-mismatch close to the 5' end, Target 4 has 6 mismatches and Target 5 is complementary, but it has a tail of 24 extra-bases in the 5' end. The hybridization process was followed by monitoring the change in the effective refractive index induced by the events.

In order to ascertain the method reproducibility, sensitivity, detection and quantification limits several amounts of Target 1 were evaluated by the same fiber sensor. This allowed the definition of the calibration curve presented as Supplementary Fig. S1 in the online version at DOI: [10.1016/j.snb.2016.04.105](https://doi.org/10.1016/j.snb.2016.04.105) with a R^2 of 0.98. The obtained results allowed us to determine the method's Detection Limit: 60 ± 20 nM and Quantification Limit: 201 ± 20 nM. These limits are quite low for a label-free technology. Indeed, even though detection limits in the hundreds of femtomolars have been reported

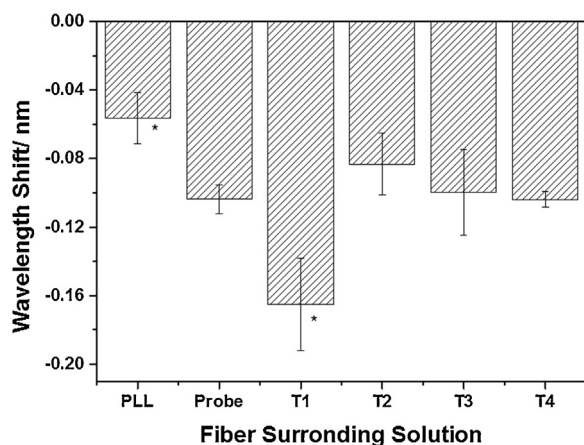


Fig. 3. Representation of the sensor response in the presence of Poly-L-Lysine (PLL), single strand DNA-probe, Target₁ (complementary), Target₂ (non-complementary), Target₃ (single mismatch) and Target₄ (six mismatches), for three independent measurements. *The values are statistically significant when compared to the Probe Signal for a $p < 0.05$.

[22–24]. All these methods required DNA labelling, increasing costs and requiring additional labelling steps. Moreover, our method also allows the quantification of the DNA in a small sample, without requiring a previous amplification process as required in Real-Time PCR assays [15] and in other biosensor based methods [22].

3.3. Analytical performance of the sensing system

In order to evaluate the applicability of the sensing system several targets were used. Each target had a peculiarity so it could be possible to ascertain if the developed system was able to detect single mismatch, if the mismatch position was relevant and if complementary sequence could be compromised by having a tail of mismatches in a 5' end.

The results showed in Fig. 3 clearly demonstrate that hybridization only occurs for Target₁, using a concentration of 0.25 μM . This result is quite interesting since it allows to conclude that our system can successfully discriminate between total-complementarity and a single mismatch. Target₄ had 6 single mismatches along the chain that could easily prevent hybridization. This result clearly shows that the method is very selective, which is reinforced by the result obtained for Target₃ which has only one mismatch. This is interesting once it can be considered as a new biosensor method for Single Nucleotide Polymorphism (SNP) detection [22,25], which can be applied in a wide range of research areas, from diagnose, forensic, genotyping, among others. Another advantage is that it does not require the development of complicated probes, e.g. Peptide Nucleic Acid (PNA), Locked Nucleic Acid (LNA), nor the use of signal amplification post hybridization process, e.g. Surface Ligation Reaction [22].

To help establish the use of this method in a real sample, a suitable amount of DNA was extracted from *V. vinifera*. This is a widely cultivated fruit crop with a harvested area above seven million hectares and more than 60 million tons of grapes produced per year. It is a diploid species with nineteen chromosomes, with a genome size of around 500 Mbp. Additionally, Target₅ was used, and although its sequence is complementary it has a tail of 24 bases on the 5' end, simulating interference of non-complementary sequences surrounding the complementary sequence, present in real DNA samples. Nevertheless, Target₅ only had a tail in one of the sequence end. As it can be seen by the analysis of Fig. 4, there is a statistical significant difference between the signal obtained for the ssDNA and Target₅. This result suggests that the sensing system

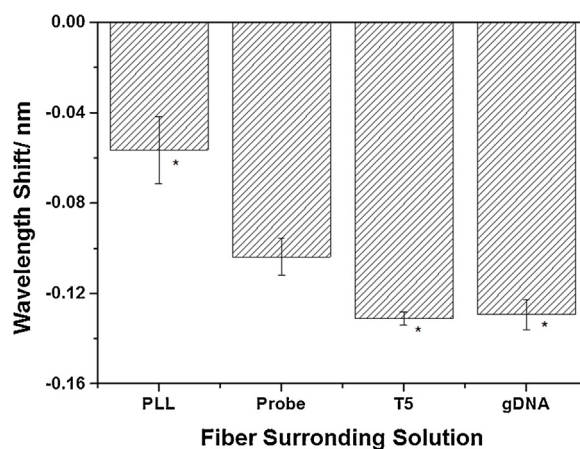


Fig. 4. Representation of the sensor response in the presence of Poly-L-Lysine (PLL), single strand DNA (Probe), Target₅ (complementary with a 24 bases tail in 5'-end) and Genomic DNA (DNA extracted from *Vitis vinifera* L.), for three independent measurements. *The values are statistically significant when compared to the Probe Signal for a $p < 0.05$.

identified this target as complementary to the ssDNA immobilized in the fiber.

The procedure applied to analyze the genomic DNA extracted from *V. vinifera* was very similar to the one used for the previous targets. However, since the DNA was still double stranded a denaturation process was applied. This was accomplished by a simple heating of the DNA sample. In order to evaluate the competitive mechanism between the immobilized probe and the two single-stranded DNA chains from the *V. vinifera* DNA sample, they were both put into contact with the LPG surface. The response observed for this sample (Fig. 4) clearly demonstrates that despite the competitive existent due to the presence of complementary sequence, the designed system can indeed detect the hybridization process.

When comparing the signal differences between the probe, Target₁ and the real DNA sample it is possible to ascertain the impact that the competitive mechanism has on the system. The hybridization of Target₁ results in a signal increase of approximately 59% on the other hand when there is a competitive mechanism between the immobilized probe and the two complementary chains of the real sample, the hybridization is followed by a 20% signal increase. As such, it is possible to say that our system is able to successfully detect the DNA in a more complex matrix (real sample). Moreover, even though we are dealing with a real sample, it is possible to see that the results are quite reproducible—the highest standard deviation in three consecutive measurements is 2%.

As all the experiments were conducted using the same molarity, 0.25 M, the number of estimated copies between the synthetic targets and the real sample are significantly different, being 5.330×10^{16} for Target₁, and 2.687×10^6 for the real sample. This emphasizes the sensibility of the method when using direct real DNA samples, and the fact that no previous amplification step was required, increasing the target number in the solution as is required for some actually used DNA-based biosensors [22]. This is an interesting feature as some DNA samples present PCR-inhibitors [15], limiting their use for genotyping procedures.

4. Conclusions

The optical DNA-based biosensor tested proved to be highly specific when synthetic samples were used, detecting a unique SNP difference. The amazing outcome was the fact that this biosensor was successfully applied in a complex matrix, *V. vinifera* genomic DNA, even when the sequence copy number is considerably lower than the synthetic target used and with the presence of

competitive mechanisms. This system makes it possible to detect and quantify DNA in real samples, using standard optical telecommunication technology, high with specificity, since the method allows the detection at a SNP level, which is why we expect that it would be quite useful in areas where the DNA detection/quantification is the main goal.

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References

- [1] X.H. Fang, W. Tan, Imaging single fluorescent molecules at the interface of an optical fiber probe by evanescent wave excitation, *Anal. Chem.* 71 (1999) 3101–3105.
- [2] S. Weiss, Fluorescence spectroscopy of single biomolecules, *Science* 283 (1999) 1676–1683.
- [3] A.K. Lockley, R.G. Bardsley, DNA-based methods for food authentication, *Trends Food Sci. Tech.* 11 (2000) 67–77.
- [4] X. Zhao, R. Tapeç-Dytioco, W. Tan, Ultrasensitive DNA detection using highly fluorescent bioconjugated nanoparticles, *J. Am. Chem. Soc.* 125 (2003) 11474–11475.
- [5] L.M. Reid, C.P. O'Donnell, G. Downey, Recent technological advances for the determination of food authenticity, *Trends Food Sci. Tech.* 17 (2006) 344–353.
- [6] X. Sun, M. Jia, L. Guan, J. Ji, Y. Zhang, L. Tang, Z. Li, Multilayer graphene-gold nanocomposite modified stem-loop DNA biosensor for peanut allergen-Ara h1 detection, *Food Chem.* 172 (2015) 335–342.
- [7] A. Uygun, DNA hybridization electrochemical biosensor using a functionalized polythiophene, *Talanta* 79 (2009) 194–198.
- [8] J. Wang, D. Xu, R. Polsky, Magnetically-induced solid-state electrochemical detection of DNA hybridization, *J. Am. Chem. Soc.* 124 (2002) 4208–4209.
- [9] L. He, M.D. Musick, S.R. Nicewarmer, F.G. Salinas, S.J. Benkovic, M.J. Natan, C.D. Keating, Colloidal Au-enhanced surface plasmon resonance for ultrasensitive detection of DNA hybridization, *J. Am. Chem. Soc.* 122 (2000) 9071–9077.
- [10] G.M. Makrigiorgos, S. Chakrabarti, Y.Z. Zhang, M. Kaur, B.D. Price, A PCR-based amplification method retaining the quantitative difference between two complex genomes, *Nat. Biotechnol.* 20 (2002) 936–939.
- [11] M. Safdar, Y. Junejo, Development and validation of fast duplex real-time PCR assays based on SYBER Green fluorescence for detection of bovine and poultry origins in feedstuffs, *Food Chem.* 173 (2015) 660–664.
- [12] S. Uno, D. Tiwari, M. Kamiya, Y. Arai, T. Nagai, T. Urano, A guide to use photocontrollable fluorescent proteins and synthetic smart fluorophores for nanoscopy, *Microscopy* 64 (4) (2015) 263–277.
- [13] I.L. Medintz, H.T. Uyeda, E.R. Goldman, H. Mattoussi, Quantum dot bioconjugates for imaging, labelling and sensing, *Nat. Mat.* 4 (2005) 435–446.
- [14] X. Zuo, F. Xia, Y. Xiao, K.W. Plaxco, Sensitive and selective amplified fluorescence DNA detection based on exonuclease III-aided target recycling, *J. Am. Chem. Soc.* 132 (2010) 1816–1818.
- [15] P. Martins-Lopes, S. Gomes, L. Pereira, H. Guedes-Pinto, Molecular markers for food traceability, *Food Technol. Biotech.* 51 (2) (2013) 198–207.
- [16] J.D. Suter, I.M. White, H. Zhua, H. Shi, C.W. Caldwell, X. Fan, Label-free quantitative DNA detection using the liquid core optical ring resonator, *Biosens. Bioelectron.* 23 (2008) 1003–1009.
- [17] K.M. Sefc, F. Regner, E. Turetschek, J. Glössl, H. Steinkellner, Identification of microsatellite sequences in *Vitis riparia* and their applicability for genotyping of different *Vitis* species, *Genome* 42 (1999) 367–373.
- [18] H.M.R. Gonçalves, L. Moreira, L. Pereira, P. Jorge, C. Gouveia, P. Martins-Lopes, J.R. Fernandes, Biosensor for label-free DNA quantification based on functionalized LPGs, *Biosens. Bioelectron.* (2015), <http://dx.doi.org/10.1016/j.bios.2015.10.001> (Accepted).
- [19] J.J. Doyle, J.L. Doyle, A rapid total DNA preparation procedure for fresh plant tissue, *Focus* 12 (1990) 13–15.
- [20] M.I. Zibaii, H. Latifi, E. Ghanati, M. Gholami, S.M. Hosseini, Label free detection of DNA hybridization by refractive index tapered fiber biosensor, *Proc. SPIE* 7715 (2010), 77151Z1–77151Z9.
- [21] F. Baldini, M. Brenici, F. Chiavaioli, A. Giannetti, C. Trono, Optical fibre gratings as tools for chemical and biochemical sensing, *Anal. Bioanal. Chem.* 402 (2012) 109–116.
- [22] K. Chang, S. Deng, M. Chen, Novel biosensing methodologies for improving the detection of single nucleotide polymorphism, *Biosens. Bioelectron.* 66 (2015) 297–307.
- [23] H. Fan, Y. Xu, Z. Chang, R. Xing, Q.J. Wang, P.G. He, Y.Z. Fang, A non-immobilizing electrochemical DNA sensing strategy with homogenous hybridization based on the host-guest recognition technique, *Biosens. Bioelectron.* 26 (2011) 2655–2659.
- [24] A.H. Wu, J.J. Sun, R.J. Zheng, H.H. Yang, G.N. Chen, A reagentless DNA biosensor based on cathodic electrochemiluminescence at a C/CxO1-x electrode, *Talanta* 81 (2010) 934–940.
- [25] W. Zhang, T. Yang, K. Jiao, Ultrasensitive indicator-free and enhanced self-signal nanohybrid DNA sensing platform based on electrochemically grown poly-xanthurenic acid/Fe₂O₃ membranes, *Biosens. Bioelectron.* 31 (2012) 182–189.

Biographies

Luis Moreira graduated with a degree in Genetics and Biotechnology and M.Sc. degree in Food Quality and Biotechnology from the University of Trás-os-Montes and Alto Douro, Portugal, in 2011 and 2013, respectively. From 2013–2015 worked in the Center of Genetics and Biotechnology, Vila Real, Portugal. He has 2 publications in SCI journals and 10 abstracts in national and international meetings.

Helena Gonçalves graduated in Chemistry, received her M.Sc. degree in Chemistry, at the University of Porto, Portugal in 2007 and 2008, respectively. In 2009 attended a post-graduation in Medical and Legal Science and in 2014 concluded her Ph.D. in Chemistry from Porto University, Portugal. She has been involved in 6 research projects. From her research work, Helena is author and co-author of 13 full-papers published SCI journals, 1 patent and several abstracts in national and international meetings.

Leonor Pereira graduated with a degree in Biotechnology Engineering at the Polytechnic Institute of Bragança, Portugal, in 2002. She obtained her M.Sc. degree in Molecular Genetics from the University of Minho, Braga, Portugal in 2006. In 2015, she finished her PhD from the University of Trás-os-Montes and Alto Douro, Portugal. From 2008–2015 she worked in the Centre of Genetics and Biotechnology, Vila Real, Portugal. She participated in 5 projects. She has 7 publications in SCI journals, 3 patents, 1 abstract published in SCI journals, 1 full-paper published in peer-reviewed journal, and 18 abstracts published in national and international meetings.

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