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Review Article

DNA-Based Platform for Halal Authentication and Combat Food Threat

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Abstract: The molecular recognition of a particular DNA target is essential not only for the development of new drugs, forensic research, and medical diagnostics, but also for the authentication of Halal status. Halal food products are those that are prepared in accordance with Islamic Shariah law while also being hygienic. Food safety plays an essential role in determining Halal foods, such as wholesome (safety, cleanliness, nutrition, and quality) food, in addition to the required Syariah regulation of halal food for Muslims. Thus, food authentication is an increasing concern and an integral part of ensuring quality foods that comply with religious faith. Furthermore, "zoonotic threats" such as bovine spongiform encephalopathy and H5N1 virus have tremendously intensified the need for foods to be safe for human health. Muslim or non-Muslim consumers will benefit from purchasing Halal foods when their rights are protected by preventing fraudulent admixing of ingredients and microbial threats. DNA-based biomarkers can recognize the distinction between different types of animal or microbial species. The detection of a particular DNA target has led to the development of a variety of different platforms such as polymerase chain reaction, molecular fluorophores and nanoparticle-based assay. This review paper is aimed at describing the DNA-based platform to

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protect the consumer's right to prevent food fraud and ensure safe Halal food.

1. Halal Food Market

"Halal" is an Arabic word that is used in the al-Quran to describe things that Muslims are allowed to do. Islamic Shari'ah law, which is based on the al-Quran (the holy book), Hadith (a collection of Prophet Muhammad's sayings), and Fiqah (the consensus opinions of a group of Islamic scholars), tells Muslims how they should eat. Muslims can eat the meat of domesticated split-hoofed animals like cattle, buffalo, sheep, goats, and camels, but they can't eat the meat of pigs or carnivorous animals like dogs (Khattak *et al.*, 2011). The world's Muslim population is growing twice as fast as the world's non-Muslim population, at a rate of 0.7% per year. The number of Muslims in the world is expected to grow by about 35% and reach 2.2 billion in the next 20 years, making up 26.4% of the world's population (Ahmed & Memish, 2019). This large number of Muslims will likely have a preference for buying products with the Halal brand name. About 16% more halal food is thought to be consumed every year, and in 2010, 63.25% of the total market value of halal food was in Asia. By 2030, more than half (1.3 billion) of the world's estimated Muslim population will live in South Asia and the Asia-Pacific region. Indonesia, Pakistan, India, and Bangladesh, which are all in South Asia and the Pacific, will make up 40% of the world's future potential halal customers. Also, by 2030, the number of Muslims will have doubled in the United States (from 5.25 million to 10.5 million) and grown by almost a third (from 44.1 million to 58.2 million) in Europe.

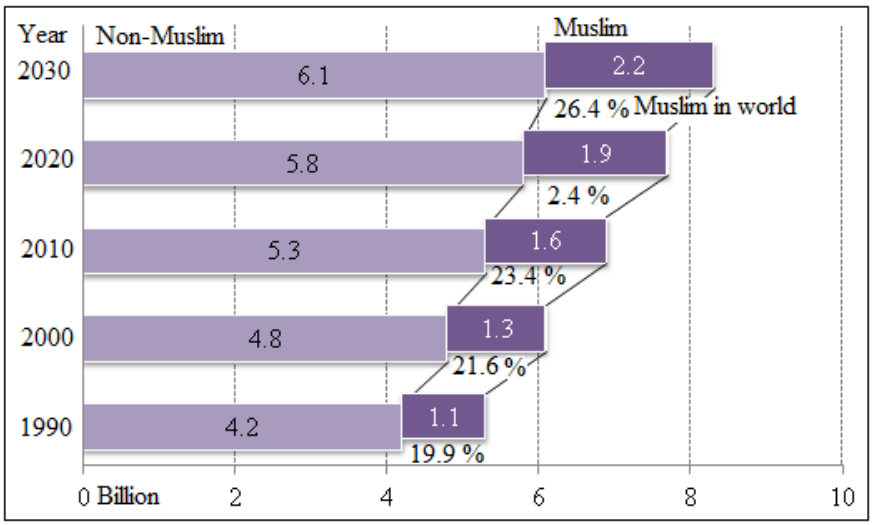

Figure 1. Prediction of potential global Muslim population by 2030 (Adapted from Pew Research, 2011).

Halal food is not only a choice for Muslims, but also for non-Muslims who want to eat safely and ethically. In the UK, there are about 2 million Muslims, but about 6 million people eat Halal meat. The market value of Halal food products in the Netherlands, including sales to non-Muslims, is estimated to be about $3 billion per year (Hughes & Malik, 2014). Even though only 20% of the world's Muslims are from Arab and Middle Eastern countries, 50% of the 10 countries with the most buying power are from these areas (Hughes & Malik, 2014).

2. Current Molecular Species Detection Techniques

The primary indicators for the molecular identification of species in foods include lipids (Rohman *et al*., 2011), proteins (Asensio *et al*., 2008), and nucleic acids (Ali *et al*., 2012). Lipid-based biomarkers for species identification in foods are based on positional analysis of fatty acid triacylglycerol (TAG) and 2-monoacylglycerol (2-MAG) (Szabó *et al*., 2007). However, lipid-based approaches have limitations in species authentication owing to changes in the spatial distribution of fatty acids with TAGs and MAGs during food processing (Ali *et al*., 2012). In several literatures, the use of protein biomarkers for animal species authentication has been documented using diverse techniques, including electrophoretic (Montowska & Pospiech, 2007), chromatographic (Chou *et al*., 2007), and spectroscopic methods (Ellis *et al*., 2005). However, protein-based techniques have been limited by the denaturation of soluble protein during heat processing of products. Furthermore, immunoassay studies of antibodies produced against a particular protein may be interrupted by cross-reactions from closely similar species, resulting in discrimination failure (Ayaz *et al*., 2006). To circumvent these constraints, DNA-based techniques have recently gained prominence in species authentication.

3. How DNA Can Combat the Threat

3.1 Short-Length DNA Biomarkers

Based on the variation of the sequence in the genome, DNA-based biomarkers offer adequate discriminating ability to distinguish variants of any animal or microbial species. Furthermore, DNA is more resistant to heat or mechanical damage than other marker compounds. Thus, DNA-based assays are promising techniques for molecular detection of pathogens in foods, bio-diagnostics, environmental monitoring, and forensic investigation. (Iwobi *et al*., 2011; Teletchea *et al*., 2005). Short-length DNA biomarkers amplify more effectively, segregate with greater resolution, and can be recovered more efficiently in degraded sample analysis. Since short DNA biomarkers are very stable under extreme conditions, they are of great

interest for use in biosensors (Jung *et al*., 2009), biochips, and forensic applications (Aboud *et al*., 2010).

3.2 DNA Biomarker-Specificity

When assessing the specificity of biomarkers, it is important to use a standard procedure to avoid confusion. The polymerase chain reaction (PCR) is a widely used molecular method for determining the type of meat present in a given food item. The method relies on the hybridization of oligonucleotide primers with a particular DNA target *in vitro*. Using an electrophoresis method, one may identify the amplified segment that belonged to a particular organism with the specific primers designed for that species. Using an agarose gel to separate the DNA fragment is the easiest PCR approach for identifying the target species. Capillary electrophoresis may be used to supplement the accuracy of the PCR-RFLP test, which relies on the restriction endonuclease digestion of PCR products to identify species (Mafra *et al*., 2008). However, the question "Is this food or ingredient what I perceive it to be?" can only be answered with a simple yes or no by conventional gel image detection of a particular target. (Woolfe & Primrose, 2004) However, there are times when knowing how much of an adulterant is present in food, or how many microorganisms pose a hazard to human health, is crucial. Using a fluorescently labelled molecule, quantitative examination of the specified target is possible with the use of real-time PCR (Wiseman, 2002).

3.3 Validation of DNA-Based Assays

To ensure the health and safety of consumers and prevent deceptive food labeling, it is crucial that high-value meat species are not partially or wholly adulterated with lower-value species in processed meals (Mafra *et al*., 2008). Meat from less desirable sources, such as canine species, may be a health hazard and a carrier for illness. Also, it has the potential to be a source of pathogens that may contaminate food. Consumers' eating habits, including vegetarianism or religious observance, may be affected by the cheaper meat being offered as adulteration. Where there is a dietary taboo against eating pork, for instance, in the religions of Judaism and Islam. However, the sensitivity and specificity of the test may be affected by the presence of numerous chemicals and inhibitors in commercial meat and food items, which may prevent the primer binding at certain locations and limit the amplification efficiency (Di Pinto *et al*., 2005). Thus, DNA-based tests are verified in both model experimental samples and in industrially processed foods like frankfurters, meatballs, burgers, nuggets, etc. Therefore, the assays may be used as laboratory methods in the food industry and by Halal food authentication authorities to safeguard consumers’ health and rights.

4. DNA-Based Species Detection Platform

4.1 Polymerase Chain Reaction PCR Assay

Polymerase Chain Reaction, or PCR for short (Mullis & Faloona, 1987), is a method for rapidly amplifying a small piece of target DNA from a heterogeneous pool of DNA in *vitro*. Through repeating cycles, PCR amplifies the target DNA segment (Kalle *et al*., 2014). PCR has emerged as a valuable and straightforward laboratory technology for authenticating Halal products by detecting a particular DNA target from a specific group of species. Thus, it has emerged as a valuable and straightforward laboratory technology for authenticating Halal products by detecting a particular DNA target from a specific group of species.

4.2 General Principles and Reaction Specificity

To detect DNA using a simple PCR experiment, oligonucleotide primers are designed to target the corresponding sequences at the ends. Primer admixture with DNA template, deoxyribonucleotides, and an appropriate buffer, followed by heat treatment, denatures the original strands of DNA. Additional cooling stimulates primer annealing, which in turn triggers DNA polymerase to create additional strands of DNA. Multiple cycles of denaturation, annealing, and polymerase activity would generate new copies of DNA. Figure 2 depicts an exponential growth in the total amount of particular target DNA with a theoretical abundance of 2n, where n is the number of cycles (Sagar, 2022).

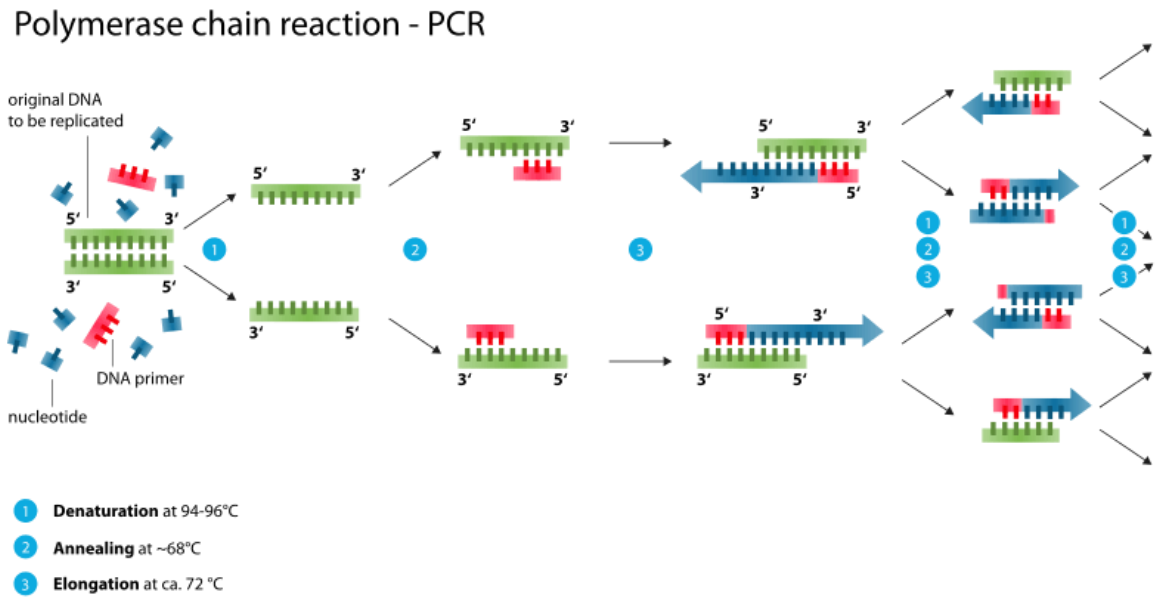

Figure 2. PCR-based replication of specific DNA targets (Source: Sagar, 2022).

4.3 Usefulness and Limitation PCR

PCR is a quick and simple technique for cloning DNA that requires only 30 cycles of denaturation, synthesis, and reannealing. A gradient PCR machine makes the process easier with the use of thermal instrumentation. Additionally, the PCR test is facilitated by the availability of computer-based primer design and oligonucleotide synthesis by many third-party service providers (Erlich *et al*., 1991).

Highly sensitive: PCR can amplify a particular DNA target from minute quantities of DNA, even from a single cell (Mollaei *et al*., 2022). Such exceptional sensitivity made it possible to use PCR for Halal verification using minute amounts of DNA from highly processed foods, even up to 0.01% of non-halal adulteration (Figure 3).

Robustness: A variety of sources of nucleic acids may be used in the PCR experiment. The appropriate templates for PCR amplification may range from pure DNAs from different species to particular sequences from materials where the DNA is severely damaged or embedded in a substrate from which conventional DNA separation is difficult. A severely degraded sample and a complex pool of DNAs may still be used by the PCR platform to recover and amplify the species-specific DNA target from a few copies to clearly detectable amounts (Rahman *et al*., 2015).

Specificity: One of the other key advantages of PCR is its high specificity, which allows for the amplification of a target DNA sequence with great accuracy and minimal background noise (Bustin *et al*., 2009). PCR relies on the use of specific primers that bind to the desired DNA sequence, minimizing the likelihood of non-specific amplification of unrelated DNA fragments (Bustin, 2010). This high level of specificity is critical in a wide range of applications, from diagnostic testing to genetic research.

However, PCR tests also have significant drawbacks. Any kind of contamination in the sample, even in tiny quantities of DNA, may yield inaccurate findings due to the high sensitivity of the PCR experiment (Smith & Osborn, 2009). Prior knowledge of the particular sequences is required to properly construct the primer for the PCR experiment. As a result, it is only relevant to species that have the required sequence information. The primers created for the PCR experiment may anneal non-specifically to non-target DNA that has similar sequences but not exactly the same ones as the target DNA (Garibyan & Avashia, 2013).

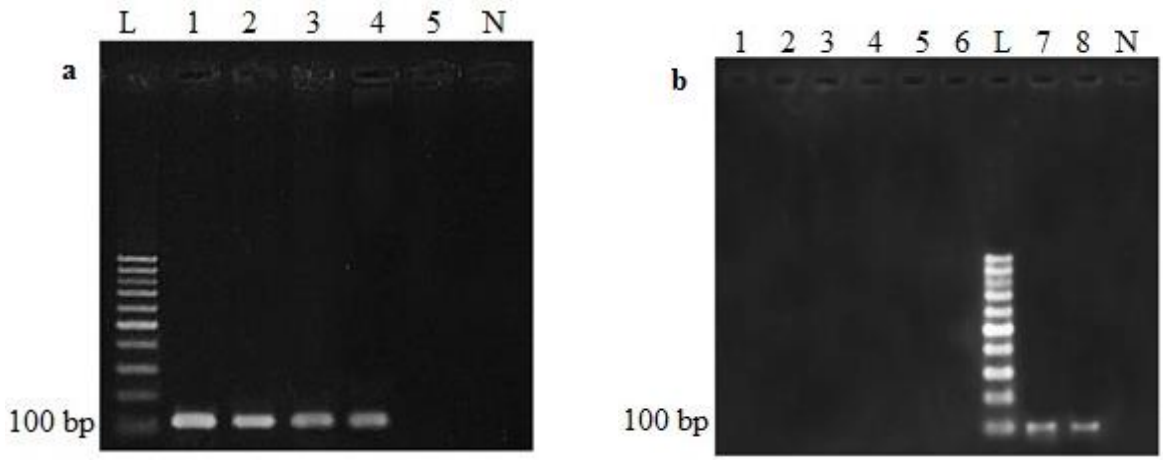

Figure 3. Halal food authentication (Frankfurter) using a mitochondrial cytb-based (100 bp) canine-specific PCR assay with 0.1% sensitivity. **A)** 100 bp PCR products obtained from 10%, 5%, 1%, and 0.1% of dog meat spiked dummy chicken frankfurters. Lane: 1 –5: 10%, 5%, 1%, 0.1%, and 0.01% dog meat spiked dummy chicken frankfurters respectively; Lane N: negative control. **b)** No PCR product was obtained from commercial frankfurters except from 0.1% dog meat spiked chicken and beef dummy frankfurters. Lane 1–3: commercial chicken frankfurters; Lane 4-6: 1–3 commercial beef frankfurters purchased from the Malaysian local market. Lane L: 100 bp Ladder; Lane 7 and 8: 0.1% spiked chicken and beef frankfurter respectively; Lane N: negative control. (Source: Ali *et al*., 2014)

4.4 Real-Time Polymerase Chain Reaction

The real-time polymerase chain reaction (RT-PCR) is an assay based on PCR that uses fluorescence signals to detect the successful amplification of a particular DNA target in a short amount of time. Fluorescent dyes or fluorescently-tagged oligonucleotide probes are used in the experiment to quantitatively or in real time measure the quantity of DNA amplified in each cycle (Rahman *et al*., 2016). This allows for the simultaneous detection and quantification of a specific DNA molecule. Innovative fluorescence chemistry and the elimination of the post-PCR end point detection system allow for real time PCR results to be obtained more quickly and consistently than those obtained using traditional PCR.

4.5 Fluorescence Measurement

The amount of DNA template used in the real-time PCR assay is connected with the fluorescence intensity (Wong *et al*., 2005). After a few cycles of the real-time run, the fluorescence intensity reaches a threshold level, depending on the DNA concentration. As a result, the fluorescence signal grows exponentially over time as the DNA templates get amplified and rise beyond background levels. However, owing to the fluorescence's saturation, it ultimately enters a plateau phase (Figure 4). Saturation fluorescence detection by the real time PCR platform is independent of the initial DNA template copy number.

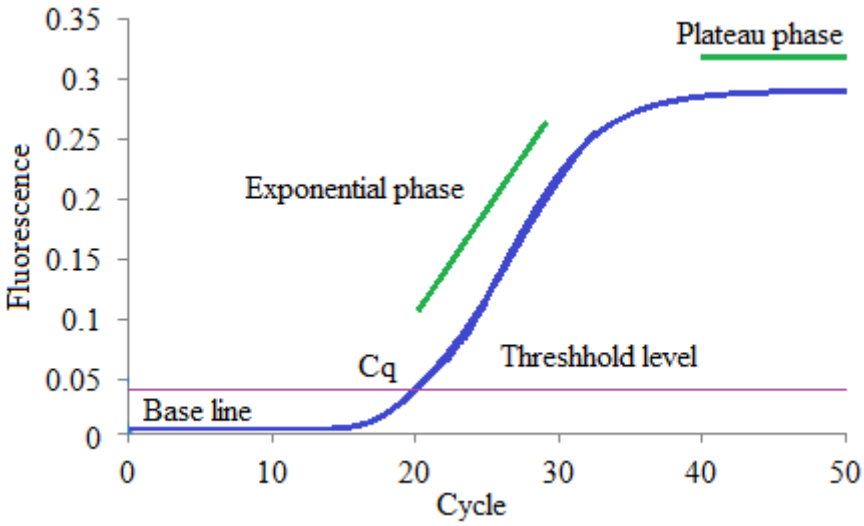

Figure 4. Fluorescence signal phase in real-time PCR.

4.6 RT-PCR Fluorescence Detection Methods

Two different kinds of chemistries in RT-PCR—dye-based chemistry, such as the SYBR-based method, and probe-based chemistry—are used to detect the fluorescence signal.

In order to detect and quantify double-stranded DNA (dsDNA), SYBR-based methods are of great value. The intercalating fluorophores or fluorescent dyes that bind only to dsDNA molecules and not single-stranded DNA (ssDNA) provide the basis for the signal amplification. The most used fluorophore dye, SYBR Green, however, may bind to all of the dsDNA in the samples, including unintended PCR products like primer dimer. The RT-PCR test also uses EvaGreen, BEBO, SYTO9, and BOXTO as fluorophores.

Particular target probe-based chemistries are based on oligonucleotide probes that have fluorescent molecules attached to them for the detection and measurement of specific targets. At the 5' and 3' ends of the probe sequence, a quencher molecule and a fluorescent dye are covalently joined. The test specificity is greatly increased by fluorescent reporter probes since they exclusively bind to complementary materials. All along the probe sequence, the closeness of the reporter and quencher dye allows for Fluorescence Resonance Energy Transfer (FRET) and the shutoff of fluorescence emission. Several reporter dyes are employed in probe-based real-time PCR assays, including 6-carboxyfluorescein (FAM), tetracholro-6-carboxyfluorescein (TET), and hexachloro-6-carboxyfluorescein, as well as quencher dyes such as 6-carboxytetramethyl-rodamine (TAMRA) or 4-(dimethylaminoazo) benzene-4-carboxylic In the PCR amplification procedure, the probe anneals with the specified target, and the DNA polymerase enzyme lengthens the target sequence. It cleaves

the probe during polymerization, allowing for an increase in fluorescence emission. TaqMan probes (hydrolysis probes) are the most commonly used proprietary probes; other types include Molecular Beacons (a hairpin probe), hybridization probes, LightUp® probes, and Solaris probes (probe with minor groove binding moeity and superbase).

4.7 Usefulness and Limitation of RT-PCR

The development of RT-PCR methods has coincided with the advent of highly efficient, automated technology (Klein, 2002). Fluorescent signal amplification during each PCR cycle allows for quantitative detection of the target DNA molecule in an RT-PCR test (Rahman *et al*., 2016). The concentration of an unknown sample may be estimated by generating a standard curve using RT-PCR values (Ct values) obtained from samples with known DNA concentrations (Ali *et al*., 2012). As a result, we may use it to quantitatively assess the extent to which food has been tampered with. RT-PCR tests coincide with a high technical sensitivity (5 copies), greater accuracy (2% standard deviation), and a large dynamic range of measurement (7-8 logarithmic decades) (Klein, 2002). Therefore, RT-PCR tests, which have a high detection probability from minute residues of distinct animal species from a diverse pool of food items, are seen as a potential molecular identification method for meat species authentication (Köppel *et al*., 2009).

However, RT-PCR assays are expensive because of the specialised equipment, reagents, and probe that are needed to get accurate quantitative findings via real-time monitoring of the growing PCR product. Additionally, the assay's increased sensitivity and shorter amplicon target it may lead to cross-species amplification in heterogeneous backgrounds (Ali *et al*., 2012).

5. NANO-Platform

In recent decades, DNA has been widely recognised as the biomolecule best suited to authenticating animal species in both raw and commercial products containing components or pathogens generated from animals. Thus, biosensors, a relatively new kind of nanoplatform with enticing nanomaterials and good physio-chemical characteristics, can be utilized for the molecular detection of DNA. The detection of analyte concentration or activity in a sample is the primary function of biosensors, which are devices that combine a bioactive material with a transducing element. Biosensors typically consist of three main parts: 1) a biologically active substance or biological recognition element or receptor; 2) a detector element or transducer; and 3) a signal processor (Mir *et al*., 2015). Thus, the first part will bind the molecule specifically to the intended target. The second part, which is

usually constructed using nanomaterials to improve the interface, will monitor the bioreceptor's response by gauging the resulting physical change and converting it into an electrical signal. The third part relays the transduced signals to a signal processor for amplification and processing. Figure 5 features the schematic for a DNA-based sensor for Halal verification. Therefore, the biological component, such as the DNA from the food, can be authenticated as Halal using the biosensor, and it will be more reliable and accurate.

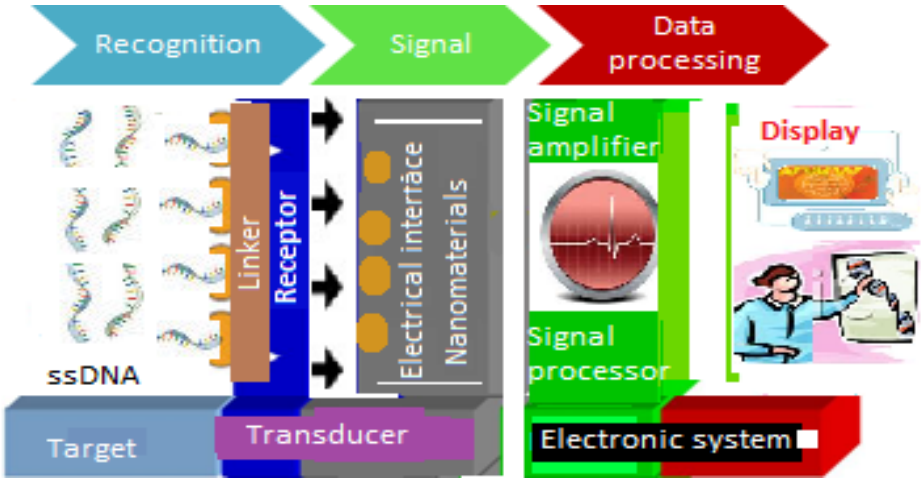

Figure 5. Halal authentication sensor based on nanoparticles.

5.1 Immobilization of Recognition Elements For Halal Authentication

For halal authentication, the most reliable biological identification components are short-target DNA probes. Therefore, the creation of more sensitive biosensors requires the immobilization of DNA using nanomaterials. Maintaining the stability of immobilized biomolecules is also important for achieving high sensitivity and selectivity. Adsorption, covalent immobilization through carbodiimide (EDC) coupling, thiol modification with nanoparticles (AuNMs), and avidin (or streptavidin)-biotin interaction are all viable options for DNA immobilization on biosensor surfaces (Ali *et al*., 2016).

5.2 Detection Techniques for Biosensor Platforms

Bioanalytical surface-enhanced Raman spectroscopy (SERS) is a rapidly emerging scientific field. SERS has great potential in bioanalysis due to its high sensitivity and excellent multiplexing (Tahir *et al*., 2021). Figure 6 shows an example of how SERS may be used for labelled and unlabelled identifications of the same short-length DNA target, expanding the scope of biomarker detection beyond its current uses in areas like Halal authentication and the detection of food-borne pathogens. In addition, optical techniques such as fluorescence,

surface plasmon resonance (SPR), chemiluminescence, colorimetry, and interferometry may also be used to identify specific targets. The detection of biomolecular hybridization has often made use of electrochemical transducers. An enzyme, ferrocene, an interacting electroactive material (a groove binder, such as Hoechst 33258), an intercalator, or nanoparticles may all serve as labels for a variety of electrochemical DNA sensors (Rashid & Yusof, 2017). Different electrochemical DNA biosensors that don't need a label have been described. Quartz Crystal Microbalance Sensors are mass-sensitive sensors that can detect even very minute changes in mass. It is made up of two electrodes and a thin disc of quartz. When an electric field is placed across an electrode, the piezoelectric material quartz deforms. The resonance frequency of a quartz crystal is proportional to the sum of the masses in motion inside it. In order to detect biomolecular interactions, a recent trend has been to mimic microcantilever detection. It relies on an effect brought on by a change in surface stress or a change in mass loading, specially surface stress variation within a cantilever resulting from the interaction between an immobilized ligand (such as a DNA probe) and an analyte (such as a DNA target).

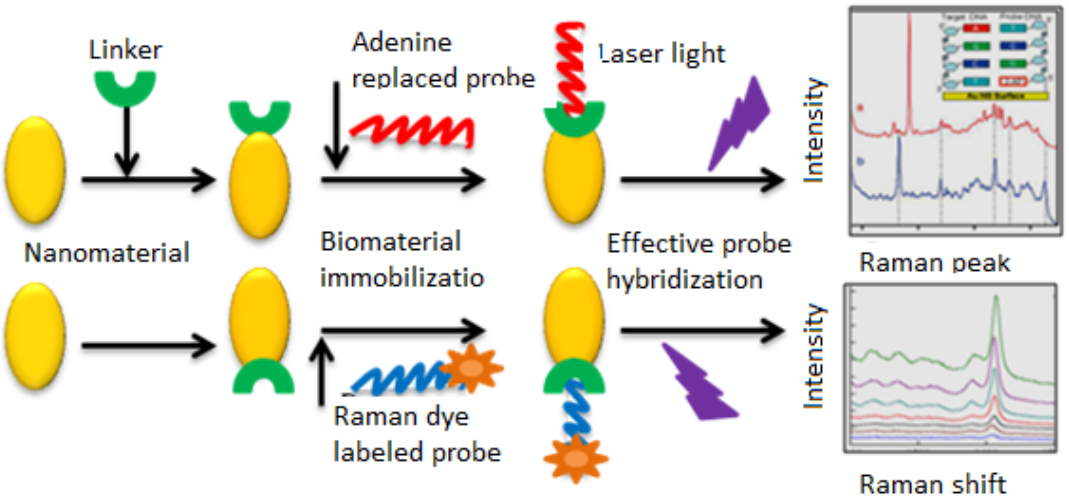

Figure 6. SERS active nanomaterials and hybridization of a particular target.

5.3 Usefulness and Limitation of Nano-platform

The necessity for expensive apparatus and reagents, as well as the photo-bleaching effect, have been detrimental to traditional DNA identification approaches based on PCR tests, particularly for quantitative analysis (Tansil & Gao, 2006). The advent of the nanoplatforms holds great hope for resolving these complex human issues with little effort and greatest efficiency. Although methods based on nanotechnology have been developed to detect DNA

for species verification in foods, these methods are still in their early stages or explanatory stages (Ali *et al*., 2012).

6. Conclusion

Despite recent developments in the identification of food pathogens and halal authentication, there are still some issues and prospects for technological advancement. The efficient and robust identification of organism species is now possible because of PCR-based DNA detection methods. Device miniaturization is possible thanks to nanobiotechnology and biosensors. To protect consumers' trust and health, improved portable, hand-held devices and biosensors for the detection of "Halal" foods may be produced via research partnerships amongst scientists in the domains of food science, engineering, molecular biology, and nanotechnology.

Author Contribution: Md. Mahfujur Rahman provided expertise in DNA-based authentication techniques, conducted critical reviews, and contributed to manuscript editing. Zaki Ahmad played a pivotal role in conceptualization and literature review. Shuhaimi Mustafa brought specialization in halal authentication to the study, conducted data analysis, and was instrumental in manuscript revision and finalization.

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Conflicts of Interest: The authors declare no conflict of interest in this work.

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