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Research paper

Species detection using HyBeacon® probe technology: Working towards rapid onsite testing in non-human forensic and food authentication applications

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ABSTRACT

Identifying individual species or determining species' composition in an unknown sample is important for a variety of forensic applications. Food authentication, monitoring illegal trade in endangered species, forensic entomology, sexual assault case work and counter terrorism are just some of the fields that can require the detection of the biological species present. Traditional laboratory based approaches employ a wide variety of tools and technologies and exploit a number of different species specific traits including morphology, molecular differences and immuno-chemical analyses. A large number of these approaches require laboratory based apparatus and results can take a number of days to be returned to investigating authorities. Having a presumptive test for rapid identification could lead to savings in terms of cost and time and allow sample prioritisation if confirmatory testing in a laboratory is required later. This model study describes the development of an assay using a single HyBeacon® probe and melt curve analyses allowing rapid screening and authentication of food products labelled as Atlantic cod (*Gadus morhua*). Exploiting melt curve detection of species specific SNP sites on the COI gene the test allows detection of a target species (Atlantic cod) and closely related species which may be used as substitutes. The assay has been designed for use with the Field Portable ParaDNA system, a molecular detection platform for non-expert users. The entire process from sampling to result takes approximately 75 min. Validation studies were performed on both single source genomic DNA, mixed genomic DNA and commercial samples. Data suggests the assay has a lower limit of detection of 31 pg DNA. The specificity of the assay to Atlantic cod was measured by testing highly processed food samples including frozen, defrosted and cooked fish fillets as well as fish fingers, battered fish fillet and fish pie. Ninety-six (92.7%) of all Atlantic cod food products, tested, provided a correct single species result with the remaining samples erroneously identified as containing non-target species. The data shows that the assay was quick to design and characterise and is also capable of yielding results that would be beneficial in a variety of fields, not least the authentication of food

1. Introduction

The application of forensic DNA techniques to non-human species is increasingly prevalent in today's legal system. It is used to support or refute prosecution or defence hypotheses in areas as wide ranging as murder [1], food safety [2], sexual assault [3] and illegal animal killing [4]. The forensic analysts in this field are routinely tasked with answering four broad questions. Firstly, *what species is present in the unknown sample?* (species identification); secondly, *how much of the species is present in the unknown sample?* (species or species' quantification); thirdly, *what area did the species originate from?* (species provenance) and finally; *what is the probability that another individual member of the same species could have left the crime scene stain?* (individual identification) [5].

Species identification is the most common question asked in non-human forensics. Techniques used to identify an individual organism to the species level are broad and include enzyme-linked immunosorbent assays (ELISA) [6], Raman spectroscopy [7], matrix-assisted laser desorption/ionization time-of-flight mass

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spectrometry (MALDI TOF) [8] and DNA-based methods [5]. DNA-based approaches are often preferred as they can offer a more robust approach. They tend to have high sensitivity due to the use of polymerase chain reaction (PCR), high specificity to the chosen target and can be used on highly processed samples, many of which have been exposed to high temperatures [9]. Common DNA techniques using PCR include PCR-restriction fragment length polymorphisms (PCR-RFLP), amplified fragment length polymorphisms (AFLP), forensically informative nucleotide sequencing (FINS), random amplified polymorphic DNA (RAPD), melt curve analyses [10,11] and DNA sequencing [12]. A lack of governance and standardisation relating to species identification in food standards means that each laboratory often develops ad-hoc approaches, many of which have been phased out of use in routine forensic applications. However, DNA sequencing is often considered the gold standard due to the ability to detect and clearly identify a large number of species specific single nucleotide polymorphisms (SNPs) in the gene regions tested [12,13].

Perhaps one of the best known uses of non-human forensic genetic techniques today is in the detection of food fraud, defined by Europol and Interpol as 'the deliberate placing on the market, for financial gain, foods which are falsely described or otherwise intended to deceive the consumer' [14]. Food authenticity and food safety testing is carried out on an international scale by a number of government and private testing laboratories [15–17]. Of recent concern is fisheries food fraud of which there are 7 distinct forms: species substitution; fishery substitution; illegal, unreported and unregulated (IUU) substitution; species adulteration; chain of custody abuse; catch method fraud; and undeclared product extension [14]. An increase in global seafood consumption has led

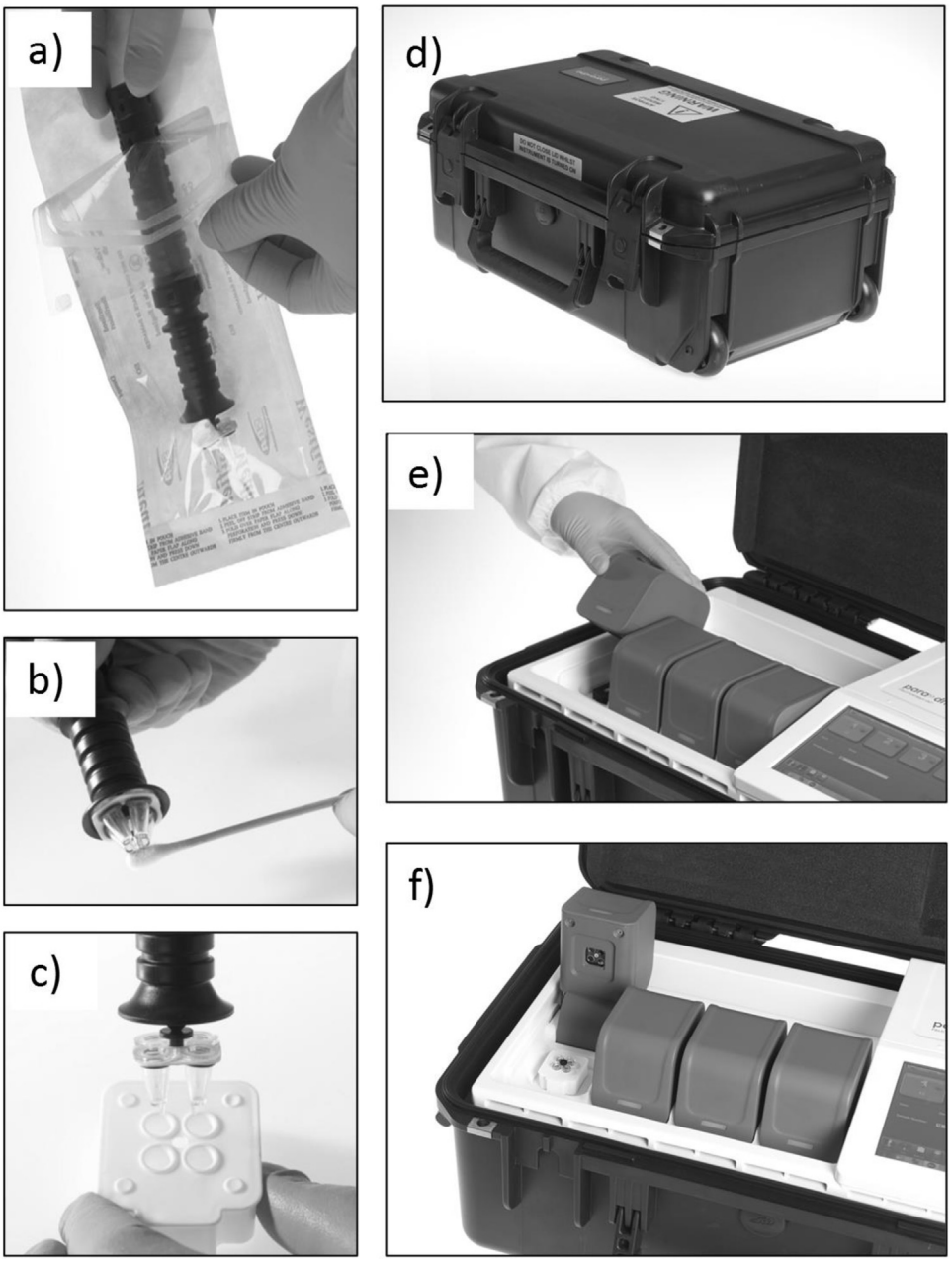

Fig. 1. To use the ParaDNA System simply, (a) open the disposable Sample Collector; (b) recover the cellular material from an evidence item; and (c) introduce the template material into the PCR plate containing the assay mix. To load the sample on to the field portable unit (d) simply, open the independent head (e) and place the PCR plate onto the heating block. The process is finished by labelling the sample, closing the head and pressing start on the touch screen.