Authenticity Methodology Working Group view on use of Next Generation Sequencing for food authenticity testing

Issue

Defra's Authenticity Methodology Working Group – Technical Sub-Group (AMWG-TSG) met on 03 July 2019 to discuss the application of Next Generation Sequencing (NGS) to food authenticity testing, with a focus on its future potential, research gaps and the need for guidance to ensure fit for purpose testing. The group took evidence from various experts in the field to help inform their discussions.

Background

NGS (or High-Throughput Sequencing (HTS)) is an umbrella term used to describe a method for sequencing genomes at high speed and at relatively low cost. It involves the sequencing of millions of fragments of DNA simultaneously (also referred to as massively parallel or deep sequencing), rather than sequentially per run as in traditional Sanger sequencing. This high-throughput process has revolutionised genomic research with its ability to sequence hundreds to thousands of genes simultaneously. Using NGS an entire human genome can be sequenced within a single day. In contrast, the previous Sanger sequencing technology, used to decipher the human genome, required over a decade to deliver the final draft.

The methodology has been most often applied in the food arena to confirm the identity of foodborne pathogens to aid outbreak investigations and to analyse microbial communities associated with foods to identify their origin. It can also be applied to the DNA of the food product ingredients themselves. More recently quantitative determination of ingredients using NGS has been explored with commodities such as herbs and spices [1-6]. However, concern has been expressed by some members of the food industry[4] who have provided evidence to show that they are receiving different and variable results from different NGS providers, which is calling into question the robustness of NGS for verification of food authenticity with regards to the quantitative assessment of ingredients.

Given the now routine application of DNA-based methods for food authenticity testing, NGS was identified by AMWG as an emerging technology of interest and a priority area for development. Defra has funded several projects to explore the feasibility of a metagenomics approach using NGS, to determine food origin (which involves analysis of genetic material obtained from environmental (bacterial) populations). A review into current uses and future applications of NGS/HTS for food authenticity testing has also been commissioned. In addition, Defra has co-funded production of an introductory e-seminar on using service providers to undertake NGS analysis http://www.foodauthenticity.uk/training

To help inform Defra on where future research efforts concerning NGS should be directed, and to explore concerns raised via the Government Chemist on the use of NGS for the quantitative identification of food ingredients, Defra convened a meeting of the AMWG-TSG and other invited experts to discuss the application of NGS to food authenticity.

Discussion

The TSG received a note produced by the Government Chemist on NGS for food authenticity and presentations on NGS from researchers, food industry NGS-users and commercial providers of NGS services to help inform their discussions. The group made the following observations:

- NGS is a valuable research tool with the potential to be widely applied to food authenticity, quality and safety analysis.
- At present, the main benefit in terms of authenticity testing appears to be for species identification of ingredients in a food product (for example using a metabarcoding approach). This has advantages over current methods due to its non-targeted approach (i.e. no need to use standards for every species of interest) and high throughput.
- Based on current information in the scientific literature and work in progress in standardisation committees, best measurement practice does not currently support the use of NGS in isolation for accurate **quantitative** analysis of food ingredients for food authenticity enforcement purpose. This has been impacted upon through issues such as amplification bias, lack of reproducibility and insufficient method validation for quantitative applications.
- When applied to **qualitative** analysis for routine food authenticity testing or enforcement analysis, NGS should be used with caution and samples should be analysed using more established confirmatory testing methods such as real time PCR to confirm results, where such methods are available.
- As with any new technology, full method validation must be undertaken to ensure fitness for purpose of the method, inclusive of looking at potential amplification bias, the presence of PCR inhibitory compounds, and processing conditions which may reduce DNA concentration.
- NGS is a powerful tool and a useful addition to the authenticity testing tool-box in the future, for example in identification of animal and plant species present in a product and in establishing traceability of products and ingredients. Further research and validation is needed to fully realise these applications and overcome some of the current barriers. Research priorities identified by the group include:
    - Working towards harmonisation and standardisation in the field of NGS analysis for food authenticity testing including:
        - Establishing fit for purpose validation approaches, setting appropriate measurement criteria and understanding assay performance characteristics.
        - Understanding of how database infrastructure, creation and maintenance impacts the fitness for purpose of NGS food authenticity testing and exploration of options to facilitate data sharing should be undertaken.
        - Guidance on critical steps in application of NGS analysis to food authenticity such as appropriate primer set design, optimising sample preparation and interpretation and reporting of results.

- Obtaining objective data to establish the scope and limitations of the quantitative capability of NGS.
- Feasibility studies to look at:
    - designing and implementing a validation exercise for a candidate metabarcoding analysis (e.g. identification of meat species ingredients by 16S metabarcoding) using a modular approach.
    - Production of appropriate reference materials for use in NGS.
    - Novel metagenomics applications for product identification (eg. Wine, fruits or other foods with an intrinsic microbial community that can potentially be exploited for verification of authenticity of the food/product).
- Further knowledge transfer activities for key NGS stakeholders in the food and drink sector (both technical and non-technical audiences). To support analysis, understanding and interpretation of NGS testing results and provide advice on when it is appropriate to apply different sequencing techniques for different food authenticity determinations e.g. screening purposes, detection of known vs. unknown targets, etc.
- Awareness and input to other UK/EU and international Working Groups examining NGS technologies in order to share and establish best measurement practice guidance.
- Engage with other funders on developing NGS capability more broadly and addressing technical barriers to improve technology transfer.

Next steps

The AMWG will work with Defra, via its Food Authenticity Programme, to develop a series of strategic research objectives to address the issues identified by the AMWG-TSG and invited experts at this meeting focusing on validation, measurement uncertainty estimation, fitness for purpose, knowledge transfer and novel research to fully realise the potential of NGS for food authenticity testing applications.

This statement reflects the current state-of-the-art with respect to NGS and food authenticity testing as of November 2020 when it was discussed and agreed by AMWG.

November 2020

References

1. Personal communication, McCormick & Company
2. A Summary of Current DNA Methods for Herb and Spice Identification Bayley: Journal of AOAC (American Association of Analytical Chemistry) International Vol. 102, No. 2, 2019
3. Overview of Analytical Tools for the Identification of Adulterants in Commonly Traded Herbs and Spices – Osman et al. : Journal of AOAC International Vol. 102, No. 2, 2019
4. European Spice Association (ESA) White Paper on Plant Metabarcoding NGS Analysis applied to Culinary Herbs and Spices, April 2019.
5. IFST (Institute of Food Science and Technology - UK) – Food Authenticity testing part 2: Analytical Techniques
6. The journal.ie Feb 2019. https://www.thejournal.ie/fsai-dna-food-scanning-tool-to-clamp-down-on-food-fraud-4499959-Feb2019/