Food authenticity

Identification of Animal and Plant Species in Food-Based Products Using Next Generation Sequencing: Results from an Interlaboratory Study

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Abstract

Purpose: An interlaboratory study involving 11 European laboratories from eight countries was conducted to support the implementation of NGS for routine food authenticity analysis. In this study the Thermo Scientific™ NGS Food Authenticity Workflow was used to determine the species composition in a range of different samples

Methods: A total of 72 samples were received by each participant. The targets included meat, fish, and plant. Each participant used the Thermo Scientific NGS Food Authenticity Workflow using the Ion Torrent™ Ion Chef™ instrument and the Thermo Scientific™ Ion GeneStudio™ S5 instrument, data was analyzed with the Thermo Scientific™ SGS™ All Species ID software. The performance of each participant was scored, and the robustness and reliability of the workflow was evaluated.

Results: The real food samples produced the most variable results which can be explained by the possible heterogeneity of the samples. Among artificial DNA mixtures, 17 of 25 meat species, 20 of 27 fish species and 17 of 25 plant species were successfully identified by all participants in all parallel samples (concordance 100%). Some of the species were identified at low concentration levels (1%).

Introduction

The complexity of the food supply chain is challenging the abilities of analytical tools used for traceability of ingredients. Although there is no reference method for food authenticity analysis, the introduction of Next Generation Sequencing (NGS) in recent years has demonstrated the suitability of this method to verify species composition of food products.

Materials and methods

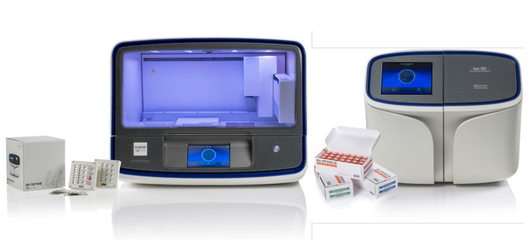
Sample preparation (Thermo Fisher Scientific)

Two types of samples were provided to the interlaboratory study participants: homogenized food samples (10 g) and DNA samples with know distribution of species. Homogenized samples were prepared by processing the food sample with Precellys homogenizer (Bertin instruments). For the DNA samples, the Thermo Scientific™ GMO extraction kit and protocol was used to extract DNA from 40-200 mg of homogenate, and DNA concentration quantified with Invitrogen™ Qubit™ Fluorometer. (Thermo Fisher Scientific).

Sample preparation (Participant location)

DNA was extracted with GMO extraction kit following the kit protocol from 40-200 mg of each homogenate, and DNA concentration quantified with Qubit Fluorometer. The DNA sample concentration was confirmed on participant site with Qubit fluorometer. Libraries for sequencing were prepared using The Thermo Scientific™SGS™ All Species Meat, Fish and Plant Analyser kits. Unique barcodes (i.e., molecular tags) were added to each sample to enable sequencing and analysis of several samples within the same sequencing run. Sample libraries were prepared for sequencing by Ion Chef Food Protection instrument and the loaded Ion chips were sequenced on GeneStudio S5 Food Protection System. Results were analyzed and reported with All Species ID Software (Thermo Fisher Scientific).

Figure 1. Ion Chef Food Protection Instrument, Ion GeneStudio Food Protection NGS System and SGS All Species ID Analyser Kits



Results

Homogenized food samples

Homogenized food sample types, names, expected content and concordance of results between participating laboratories are presented in Tables 1-3. For some species, the concordance result was not calculated (N/A) as these species were not listed as ingredients and presence or absence in the sample could not be confirmed. Each laboratory analyzed meat samples in 4 replicates and fish and plant samples in 2, all replicates and laboratories are considered when calculating concordance results. Boxplots of all results are presented in Figure 2.

Artificial DNA mixtures

DNA sample content (species and relative portion) and concordance of results between participating laboratories are presented in Tables 4-6. Each laboratory analyzed meat samples in 4 replicates and fish and plant samples in 2, all replicates and laboratories are considered when calculating concordance results. Samples with non-target species are presented in grey font. Boxplots of all results are presented in Figure 2.

Table 1. Meat products, expected content and concordance of participant results

Target	Food type	#	Sample name	Expected content and concordance
MEAT	Dry food	1	Beef jerky	Bos taurus: 100 %
		2	Risotto with chicken	Gallus gallus: 90.9 %, Bos taurus: 86.4 %
	Deep frozen food	3	Minced lamb meat	Ovies aries: 100 %, Bos taurus: 100 %
		4	Beef burgers	Bos taurus: 68.2 %
	Canned food	5	Canned ham	Sus scrofa: 86.4 %
		6	Canned chicken	Gallus : 79.5 %
	Liquid food	7	Beef soup with tomatoes	Bos taurus: 100 %
		8	Chicken soup	Gallus : 84.1 %
	Fresh raw food	9	Minced turkey meat	Meleagris gallopavo: 77.3 %, Sus scrofa: N/A
		10	Minced meat patties	Sus scrofa: 100 %, Bos taurus: 100 %

Table 2. Fish products, expected content and concordance of participant results

Target	Food type	#	Sample name	Sample content
FISH	Dry food	1	Dried haddock stockfish pieces	Melanogrammus aeglefinus: 90.9 %
		2	Dried and smoked tuna	Katsuwonus pelamis: 100 %
	Deep frozen food	3	Wild fish patties	Rutilus rutilus: 100 %, Leuciscus leuciscus: 86.4 %, Leuciscus idus: 100 %, Perca fluviatilis: 90.9 %, Katsuwonus pelamis: N/A
		4	Fish fingers	Gadus chalcogrammus: 81.8 %
	Canned food	5	Canned sardines	Sardina pilchardus: 77.3 %
		6	Canned tuna	Katsuwonus pelamis: 40.9 %
	Liquid food	7	Rainbow trout soup	Oncorhynchus mykiss: 95.5 %, Pollachius virens: N/A
		8	Salmon soup	Salmo salar: 100 %
	Fresh raw food	9	Fillet of flounder	Platichthys flesus: 90.9 %, Salmo salar: N/A
		10	Fillet of pike	Esox lucius: 100 %

Table 3. Plant products, expected content and concordance of participant results

Target	Food type	#	Sample name	Sample content
PLANT	Dry food	1	Wheat flour	Triticum aestivum: 100 %, Avena sp.
		2	Cinnamon powder	Cinnamomum zeylanicum: 40.9 %
	Deep frozen food	3	Peas, corn & bell pepper mixture	Pisum sativum: 100 %, Capsicum sp.: 95.5 %, Zea mays: 90.9 %
		4	Frozen peas	Pisum sativum: 100 %
	Canned food	5	Canned corn	Zea mays: 68.2 %, Pisum sativum: N/A, Triticum aestivum: N/A
		6	Marinated tofu	Glycine max: 100 %, Allium sativum: 81.8 %
	Liquid food	7	Spinach soup	Spinacia oleracea: 36.4 %
		8	Carrot & ginger soup	Daucus carota: 100 %, Allium cepa: 100 %, Avena sp.: 36.4 %
	Fresh raw food	9	Fresh fennel	Foeniculum vulgare: 95.5 %
		10	Fresh potato	Solanum sp./tuberosum: 86.4 %

Table 4. Artificial DNA mixtures (meat), concordance of participant results

Target	#	Sample content	% of sample	% concordance
MEAT DNA	1	Bos taurus	100	100
	2	Sus scrofa	100	100
	3	Bos taurus	33	100
		Sus scrofa	33	100
		Gallus	33	100
	4	Meleagris gallopavo	40	100
		Rangifer tarandus	40	100
		Ovis aries	20	100
	5	Rangifer tarandus	5	100
		Ovis aries	5	100
		Odocoileus hemionu/virginianus	90	100
	6	Bos taurus	1	100
		Sus scrofa	5	100
		Gallus	94	100
	7	Cervus elaphus	50	100
		Equus caballus	30	90.9
		Alces alces	20	86.4
	8	Ovis aries	99	43.2
		Cervus elaphus	1	43.2
	9	Theragra chalcogramma (fish)	45	100
		Perca fluviatilis (fish)	45	100
		Meleagris gallopavo	10	95.5
	10	Theragra chalcogramma (fish)	90	100
		Equus caballus	5	100
		Alces alces	5	100
	11	Perca fluviatilis (fish)	94	100
		Equus caballus	5	95.5
		Alces alces	1	95.5
	12	Theragra chalcogramma (fish)	99	100
		Meleagris gallopavo	1	97.7
	13	Theragra chalcogramma (fish)	100	100
	14	Perca fluviatilis (fish)	100	100

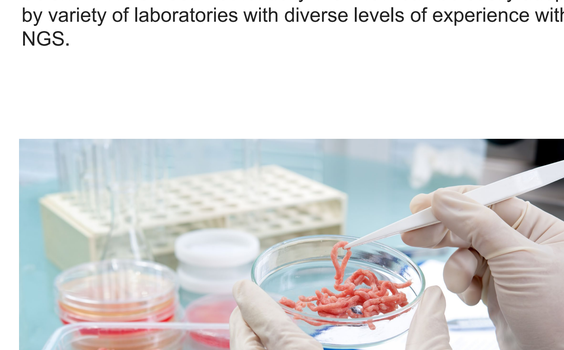
Table 5. Artificial DNA mixtures (fish), concordance of participant results

Target	#	Sample content	% of sample	% concordance
FISH DNA	15	Perca fluviatilis	100	95.5
	16	Thunnus albacares	100	95.5
	17	Oncorhynchus mykiss	33	100
		Sander lucioperca	33	100
		Coregonus muksun	33	100
	18	Sander lucioperca	40	100
		Salvelinus sp.	40	100
		Perca fluviatilis	20	100
	19	Thunnus albacares	5	100
		Perca fluviatilis	5	100
		Esox lucius	90	100
	20	Thunnus albacares	1	90.9
		Perca fluviatilis	5	100
		Esox lucius	94	100
	21	Salvelinus sp.	50	100
		Sander lucioperca	30	100
		Thunnus albacares	20	100
	22	Salmo salar	99	95.5
		Sander lucioperca	1	90.9
	23	Salmo salar	45	100
		Platichthys flesus	45	100
		Gadus chalcogrammus	10	68.2
	24	Bos taurus (meat)	90	100
		Thunnus albacares	5	100
		Esox lucius	5	100
	25	Sus scrofa (meat)	94	100
		Thunnus albacares	5	100
		Esox lucius	1	95.5
	26	Bos taurus (meat)	99	100
		Thunnus albacares	1	100
	27	Bos taurus (meat)	100	100
	28	Sus scrofa (meat)	100	100

Table 6. Artificial DNA mixtures (plant), concordance of participant results

Target	#	Sample content	% of sample	% concordance
PLANT DNA	29	Thymus vulgaris	100	86.4
	30	Coriandrum sativum	100	100
	31	Thymus vulgaris	33	81.8
		Ocimum basilicum	33	100
		Rosmarinus officinalis	33	100
	32	Rosmarinus officinalis	40	95.5
		Coriandrum sativum	40	100
		Allium sativum	20	100
	33	Allium sativum	5	100
		Origanum sp./ vulgare	5	100
		Anethum/Foeniculum graveolens	90	100
	34	Allium sativum	1	90.9
		Rosmarinus officinalis	5	100
		Ocimum basilicum	94	95.5
	35	Allium sativum	50	90.9
		Origanum sp./ vulgare	30	100
		Anethum/Foeniculum graveolens	20	100
	36	Coriandrum sativum	99	95.5
		Allium sativum	1	81.8
	37	Oncorhynchus mykiss (fish)	45	100
		Salmo salar (fish)	45	100
		Origanum sp./Origanum vulgare	10	100
	38	Oncorhynchus mykiss (fish)	90	100
		Rosmarinus officinalis	5	100
		Anethum graveolens	5	100
	39	Salmo salar (fish)	94	100
		Coriandrum sativum	5	100
		Anethum graveolens	1	100
	40	Oncorhynchus mykiss (fish)	99	100
		Coriandrum sativum	1	100
	41	Oncorhynchus mykiss (fish)	100	100
	42	Salmo salar (fish)	100	100

Figure 2. Result concordance percentage for each sample type



Conclusions

Variance between laboratories was higher with real food samples, which is congruent with sample complexity and workflow steps performed by participant laboratories.

- 76 % of food sample species and 96 % of species in DNA samples reached over 80% concordance between replicates and laboratories.
- 70 % of species were correctly identified with every sample replicate with every laboratory

Results indicate that careful upstream sample processing is a key element in species identification process to ensure robust and reliable results. Interlaboratory study showed that the Thermo Scientific NGS Food Authenticity Workflow can be easily adopted by variety of laboratories with diverse levels of experience with NGS.

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