Report on the DNA Analysis of Samples Submitted by CBC Marketplace

Information
The items (listed in Items Receipt 1 section) submitted to the NRDPFC by CBC Marketplace, in July 2016, consisted of menu items, containing cooked chicken meat, from local fast-food chain restaurants.

Additional a second set of samples was collected in December of 2016 (listed in Items Receipt 2). The second set of samples was collected from various Subway shops and processed.

Purpose
At the request of CBC Marketplace, the items (listed in the Items Receipt sections) were analyzed to measure the quantities of chicken (*Gallus gallus*) and non-chicken DNA, and to estimate the quantities of meat-derived protein and non-meat-derived protein.

Items Receipt 1
The following items were received July 2016:

Item	Restaurant	Description Of Item	Description of Meat	Laboratory ID
1	A&W	Grilled Chicken Sandwich	Individual Patty	MPAW01
2	A&W	Grilled Chicken Sandwich	Individual Patty	MPAW02
3	McDonald's	Grilled Chicken Sandwich	Individual Patty	MPMC01
4	McDonald's	Grilled Chicken Sandwich	Individual Patty	MPMC02
5	Subway	Submarine Sandwich	Formed Patty	MPSW01
6	Subway	Submarine Sandwich	Formed Patty	MPSW02
7	Subway	Submarine Sandwich	Cut/diced Pieces	MPSW03
8	Tim Horton's	Pita Wrap	Cut/diced Pieces	MPTH01
9	Tim Horton's	Pita Wrap	Cut/diced Pieces	MPTH02
10	Wendy's	Grilled Chicken Sandwich	Individual Patty	MPWD01
11	Wendys	Grilled Chicken Sandwich	Individual Patty	MPWD02

Items Receipt 2
The following were received December 2016:

Item	Restaurant	Description of Item	Description Of Meat	Laboratory ID
1	Subway	Submarine Sandwich	Formed Patty	191B1
2	Subway	Submarine Sandwich	Strip	191B2
3	Subway	Submarine Sandwich	Formed Patty	200F1
4	Subway	Submarine Sandwich	Strip	200F2
5	Subway	Submarine Sandwich	Formed Patty	287K1
6	Subway	Submarine Sandwich	Strip	287K2
7	Subway	Submarine Sandwich	Formed Patty	1535W1
8	Subway	Submarine Sandwich	Strip	1535W2
9	Subway	Submarine Sandwich	Formed Patty	1620B1

10	Subway	Submarine Sandwich	Strip	1620B2

Upon receipt, all items were stored in Freezer #12 in the Tissue Storage Room and Freezer #9 in the NRDPFC automation laboratory.

Examination

Test #1

Chicken samples were collected from A&W, McDonalds, Subway, Tim Horton's, Subway and Wendy's.

Each sample was sub-sampled 3 times and tested separately for chicken DNA and plant DNA. We were able to quantify the relative amounts of chicken vs. plant filler in these samples through PCR amplification. All of the samples had significant amounts of chicken DNA amplification, which was confirmed by DNA sequencing. Only the subway samples had significant plant DNA amplification, and the DNA sequencing results for these were a match to soy. The non-subway samples do not appear to have a significant quantity of plant DNA. Based on the low quantity of plant DNA of the non-Subway samples, we could not continue the analysis on these samples.

Test #2

A second batch of Subway samples were collected and tested for bird and plant DNA. We were able to determine the relative amounts of bird vs plant DNA within each sample. The original set of subway samples were also re-run to be sure that we were consistent across both tests. The bird DNA was determined to be chicken and the plant DNA was determined to be soy product.

Notes

- The second test of the non-subway samples yielded basically the same results and we were unable to determine if a specific plant filler was present, as the amounts of plant DNA were very low in them.
- The second batch of subway samples were sampled only 1 time each instead of 3 due to time constraints. The samples may have more variation in them, as each portion of a sample may contain a different ratio of chicken/plant filler.
- Each quantification of plant and chicken DNA was repeated in both tests were performed at 5 different DNA dilutions and the results averaged to determine a final percentage. This helps control against technical errors and increases the accuracy of the test
- We are unable to determine the exact soy product that is contained within the chicken.

Summary of Results

The following tables show the final results of the products in question. Table 1 contains the initial samples from July 2016. Table 2 contains the samples collected in December 2016. Each table compares the relative amount of chicken DNA vs the amount of plant DNA. A biopsy punch was used to take an approximately 30mg portion from the interior of each sample. The first set of samples were sub-sampled three times to control for potential uneven distributions of plant filler and chicken within each sample.

Most of these samples contained a percentage of chicken DNA that was in the mid 80's to low 90's. We ran the samples on the ABI3730 sequencer to try and determine the origin of the plant DNA but were unable to determine any signficant potential filler for these samples. This can be caused by there not being enough of a single plant species' DNA; a mixture of different plants present in low quantities will not provide a clean sequence without additional testing for individual species separately.

The samples that we received from Subway had a much higher plant DNA percentage than the other samples. There was enough DNA for us to sequence the product and determine that these samples contained a substantial amount of soy DNA. The subway samples originally provided we tested a second time along with the second set of subway samples to confirm the results.

Table 1

Item ID	Sub-sample	Chicken (*Gallus gallus*) DNA Proportion (%)	Plant DNA Proportion (%)	Standard Deviation
MPAW01	*A*	88.1	11.9	2.7
	B	90.2	9.8	1.7
	C	91.4	8.6	1.8
MPAW02	*A*	88.9	11.1	0.4
	B	89.5	10.5	1.1
	C	88.4	11.6	1.7
MPMC01	*A*	83.1	16.9	6.5
	B	79.9	20.1	8.2
	C	88.6	11.4	2.8
MPMC02	*A*	84.4	15.6	5.9
	B	85.6	14.4	6.0
	C	88.2	11.8	3.5
MPSW01	*A*	62.2	37.8	11.8
	B	61.6	38.4	13.5
	C	68.7	31.3	11.9
MPSW02	*A*	61.8	38.2	14.8
	B	61.9	38.1	14.2
	C	58.0	42.0	12.6
MPSW03	*A*	58.1	41.9	12.1
	B	89.2	10.8	1.0
	C	45.6	54.4	7.4
MPTH01	*A*	84.7	15.3	2.9
	B	86.2	13.8	3.2
	C	88.6	11.4	1.2
MPTH02	*A*	87.3	12.7	1.0
	B	84.9	15.1	1.6
	C	87.4	12.6	4.6
MPWD01	*A*	88.5	11.5	4.5

	B	89.1	10.9	8.0
	C	86.8	13.2	11.8
MPWD02	*A*	89.4	10.6	1.7
	B	89.9	10.1	0.7
	C	87.8	12.2	4.5

Table 2

Item ID	Sub-sample	Chicken (Gallus gallus) DNA proportion (%)	Plant DNA Proportion (%)	Standard Deviation
191B	1	47.6	52.4	17.5
	2	36.6	63.7	13.0
200F	1	48.3	51.7	12.5
	2	34.7	65.3	7.7
287K	1	38.7	61.3	5.1
	2	38.5	61.5	8.2
1535W	1	70.3	29.7	17.3
	2	27.6	72.4	17.8
1620B	1	44.7	55.3	7.9
	2	44.6	55.4	29.3

Appendix: Technical Section

DNA (deoxyribonucleic acid):

Living organisms are made of cells and within most of their cells are chromosomes made up of deoxyribonucleic acid (DNA). DNA is the blueprint that encodes all the materials needed for the development and function of each unique organism.

Strands of DNA are made up of four different building blocks known as nucleotides. A DNA sequence can be determined by knowing the order in which these nucleotides are arranged. While the majority of the nucleotide sequence does not vary between organisms, small portions vary significantly enough that identification of species and individuals within a species can be carried out.

DNA Extraction and Quantification:

DNA was extracted from the samples using a MagneSil Max-Yield (Promega) extraction protocol. Extracted DNA from the case items was quantified using a fluorometer-based PicoGreen (Invitrogen) assay on the BMG FluoStar Galaxy 96-well plate system. The DNA quantification test is sensitive to 1ng of DNA. Relative plant and chicken DNA were quantified by separate PCR amplification of a universal plant chloroplast primer and a universal vertebrate mitochondrial DNA primer. PCR amplifications where performed at 5 different input DNA quantities and the resulting amplicons DNA quantities were measured by agarose gel electrophoresis stained with Ethidium Bromide and a PicoGreen assay.

Species Identification:

For species identification a region of the cytochrome oxidase I (COI) gene within the mitochondrial DNA was amplified using primers designed to amplify most bird species.. Another primer set for a region of the trnL intron within plant chloroplast DNA designed to amplify most plant species These amplifications are sensitive to 10pg of DNA. The amplification products were separated on an agarose gel to visualize results. If amplified DNA was produced, it was then sequenced on an ABI 3730 DNA Analysis System.

These sequences were then compared to several cytochrome oxidase I or trnL sequences respectively, originating from control sequences of closely related species. Phylogenetic analysis was subsequently carried out to show which of the sequences within the database was most closely related to those from the samples, thus indicating the species of origin. Sequences were analysed using the software programs Sequence Analysis 5.4 and the phylogenetic software package MEGA 6 and the NCBI BLAST database (https://www.ncbi.nlm.nih.gov/).

Standard Deviation:

Samples were quantified at multiple dilutions of input DNA quantities to improve the accuracy of the testing results. Because the PCR amplifications are sensitive to very low quantities of DNA, multiple tests with decreasing DNA quantities ensuring the measurements are within the detection limits of the assays used. This also provides a measure of variation between multiple tests that may result from technical issues.