United States Department of Agriculture

Food Safety and Inspection Service

MLG 20

LCD-Macroarray Method for Species Identification in Meat and Poultry Products

This method describes the laboratory procedure for performing Species Identification on meat and poultry products using a LCD-Macroarray method to identify multiple species in a single test.

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Introduction

The LCD-Macroarray procedure described in this section is based upon principles established previously by Iwobi et al., 2011, and modified for commercial application and use by Chipron, GmbH, Berlin, Germany. This technology is notable for its ability to detect low level adulterants within food samples and detection of multiple different species from one sample with a single test. The technology works by utilizing a universal primer system to amplify a small genomic area (125-165 bp) of vertebrate 16S rRNA with conserved outer regions and an internal hypervariable region that can be detected by capture probes. Biotinylated PCR products of this region are generated from nucleic acid extracts of food samples. The labeled amplicons are then hybridized to the species-specific capture probes immobilized on the macroarray chip surface. After a short wash routine, visualization of positive samples is mediated by substrate cleavage from streptavidin tagged horse radish peroxidase (HRP) to create a visible color change that is analyzed by imaging software. An outline of this process is shown in Figure 1. It should be noted that this assay works regardless of the state of the meat, cooked or raw, and relies on the ability to obtain intact DNA from the sample. This test was first adapted and used in FSIS to detect Feline and Canine tissue pursuant to S. 3042, Title XII, subtitle E, section 125 of the 2018 Agriculture Improvement Act making the slaughter of these animals illegal for human consumption.

The methods described in this guidebook are for use by the FSIS laboratories. FSIS does not specifically endorse any of the mentioned test products and acknowledges that equivalent products may be available for laboratory use.

KEY DEFINITIONS

LCD-Macroarray – A Low-density Chip DNA (LCD) Macroarray is a collection of macroscopic DNA spots, each representing a small oligonucleotide sequence complementary to a species-specific sequence of genomic DNA, arrayed on a solid slide by covalent attachment.

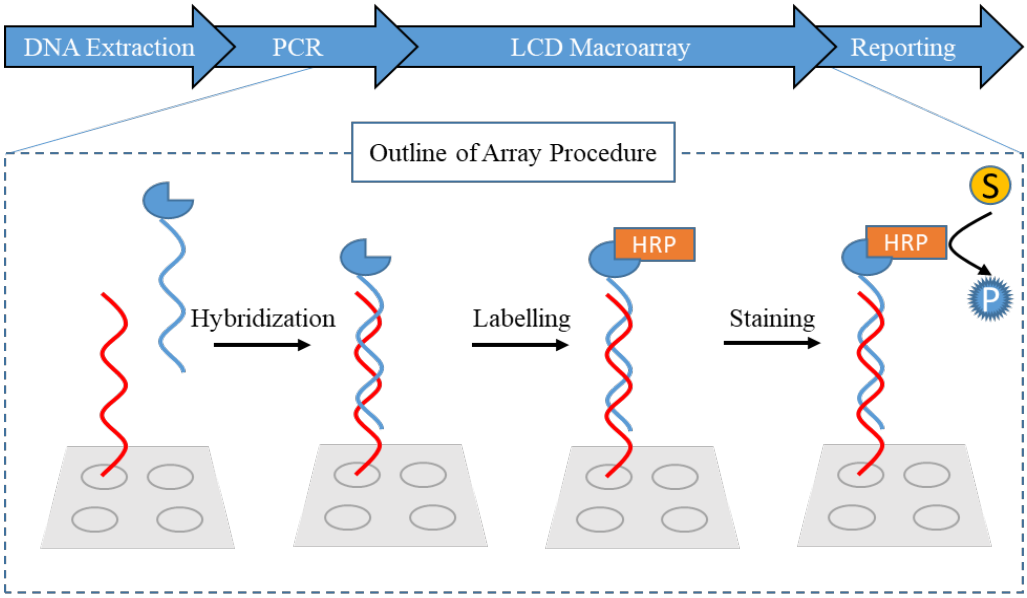

Figure 1. Overview of LCD-Macroarray procedure. DNA is extracted from food samples and then amplified by PCR using biotinylated primers. PCR amplicons (blue) are then hybridized to the LCD chip surface to species-specific capture probes (red). Streptavidin-horse radish peroxidase conjugate (HRP) is then bound. Visualization occurs through staining with HRP substrate (S) that is converted to a dark precipitate (P).

Safety Precautions

The Safety Data Sheet (SDS) may be obtained from the manufacturer for the media, chemicals, reagents, and microorganisms used in the analysis. The personnel who will handle the material should read the SDS prior to startup.

QUALITY CONTROL

Lab Quality Control Procedures

Quality control for each lot is confirmed by running known species tissue and purified water through the entire method as a positive and negative control, respectively. Any species detectable by the USDA 1.0 LCD chip can be used.

All kit components should be stored at the appropriate stated temperature when not in use, to preserve and maintain all components. Observe the manufacturer's expiration date of all test kit components. Kits should not be used beyond the expiration date. Do not mix components from one lot with components from another lot.

The test sample used for extraction must be representative of the entire original sample in order to ensure that test results accurately reflect the true composition of the original sample.

The working principle for this method is DNA hybridization, and so the specificity and sensitivity of the entire method is controlled during the 30-minute hybridization step. This means that any deviation during this step can result in non-specific binding (leading to false positives) or no binding (leading to false negatives). The two greatest factors during this step are temperature and buffer composition. It is critical to work in a waterbath that is preheated to the desired temperature prior to use and that maintains uniformity. It is also highly critical to precisely control pipetting volumes with calibrated pipettes to maintain proper buffer concentrations. **Deviations of more than 1°C or 1 µl may cause false results.**

All components (kit buffers, chips, and chamber) should be equilibrated to room temperature prior to use.

When using tissue homogenizer for sample preparation, be sure to clean probe before and after use, to ensure contamination between samples does not occur. See sample preparation section for more details.

Equipment, Kits, Reagents and Supplies

Table 1: Equipment and Kits for Species Identification

Equipment	Supplier	Purpose
Standard Thermocycler		PCR Amplification
LCD-Array CHIP-Scanner	Chipron, CHIP-Scanner PF7250U	Analysis of array chip
Benchtop Centrifuge with slide adapter	Chipron, FVL2400N	Drying of array chip
Humidity Chamber	Chipron	Containment of array chip during hybridization
Waterbath, calibrated to 36ºC ± 1ºC	General Lab Supplier	Hybridization of target
Microcentrifuge with 1.5ml tube rotor	General Lab Supplier	DNA Extraction
Small Homogenizer (optional)	General Lab Supplier	Mixing of sample
Analytical balance	General Lab Supplier	Weighing of sample, Pipette verification
Qiagen DNeasy Blood and Tissue Kit	Qiagen, Catalog #69504	Extraction and isolation of genomic DNA
HotStar Taq Polymerase Mastermix	Qiagen, Catalog #203443	DNA amplification
USDA 1.0 LCD-Array Kit A	Chipron, Catalog #A-200-12, LCD-Array Kit USDA 1.0	Macroarray chip

Table 2: Reagents and Supplies

Reagent	Supplier	Purpose
95-100% Ethanol	General Lab Supplier	DNA Extraction
De-ionized ultrapure or PCR Grade water (ddH_2O)	General Lab Supplier	PCR Amplification
Physiological saline solution (optional)	General Lab Supplier	Homogenization
Control species tissue		Experimental Control
1.5 ml centrifuge tubes	General lab Supplier	Extraction and storage of DNA
1000ul, 200ul and 20ul pipettors with tips	General Lab Supplier	Add and mixing of reagents
0.2 ml PCR tubes	General Lab Supplier	PCR Amplification
Small dishes or containers	General Lab supplier	Washing of array slides
Small weigh boats	General Lab Supplier	Weighing sample
70% isopropyl alcohol or ethanol (optional)	General Lab Supplier	Homogenizer cleaning
1N sodium hydroxide (optional)	General Lab Supplier	Homogenizer cleaning

METHOD

Sample Prep

All types of product samples are prepared as follows:

a. Using standard practices, weigh out and collect 30 ± 5 mg of sample into a labelled tube.

b. If the sample is not homogeneous, blend sample as finely as possible with a commercial blender or tissue homogenizer. Homogenization can help when dealing with meat mixtures such as coarsely ground sausages to ensure a representative mixture of all species is present in the final sample.
   - Before and after use, clean homogenizer with 1N NaOH, then 70% isopropanol, and finally rinse with ddH_2O to eliminate any residual DNA transfer between samples.
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c. If necessary, samples may be held at this point, frozen (-20°C or below) before use.

DNA Extraction

DNA is extracted from prepared sample using commercially available kits, following manufacturer's instructions. This method was validated using the DNeasy Blood & Tissue Kit (Qiagen), using provided protocol titled "Purification of Total DNA from Animal Tissues (Spin-Column Protocol)". DNA is eluted in 200 µl elution buffer. (Note: The use of 30 ± 5 mg tissue was optimized for this kit.)

PCR Amplification

PCR amplification is completed for each sample, a positive DNA control (from extracted tissue) and a no template control (PCR water). Any species present on the USDA 1.0 chip can be used as a positive control.

PCR amplification of sample DNA is carried out as follows:

Reaction mixtures:	12.5 µl of 2x Hotstar Taq Master Mix or equivalent	
	1.5 µl of Primer mix (MEAT, included with Chipron kit)	
	6 µl of PCR grade water	
	5.0 µl of target sample DNA	
	25 µl Total	
Cycle parameters:	1x cycle	95°C for 5 min
	35x cycles	94°C for 30 sec
		57°C for 45 sec
		72°C for 45 sec
	1x cycle	72°C for 2 min.

Resulting amplicons can be stored frozen (-20°C or below) until further analysis.

LCD-Macroarray Analysis Procedure

For sample analysis, samples should be run alongside a negative blank control and a known positive control that is present on the USDA 1.0 chip. These controls should be run on each chip.

The macroarray procedure is conducted as described in the USDA 1.0 LCD Array Kit User Manual, with the following modification: Hybridization is conducted at 36ºC instead of 35ºC. Critical information for this procedure can be found in the Quality Control section highlighted in this document.

References

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For questions, please contact askfsis@usda.gov and include “MLG20” in the subject line.