

Application News

Microchip Electrophoresis

No.B41

Identification of Contaminants (Animal Hair) Using DNA Testing

If a contaminant is discovered during the process of manufacturing foods, medicines, and cosmetics, etc., it is essential to identify the contaminant not only to clarify the cause or source of the contamination but for establishing a standard operating procedure to prevent the recurrence of such contamination as required in hygiene control.

Presumptive inspection of contaminants is typically conducted by microscopic observation, but since this requires specialized knowledge and experience, it is difficult for inexperienced individuals to make a clear assessment and to determine the source of contamination, human or animal. Especially, with respect to animal hair, a very common contaminant, it is extremely difficult to determine the type of contamination by visual inspection. Reliable

methodology is therefore required to solve this problem.

The Aichi Industrial Technology Institute Food Research Center has developed a DNA testing method for identifying the type of animal associated with specific hair contaminants^(*). The animal species that can now be detected reliably with this method include 6 types of farm animals (cow, pig, chicken, horse, sheep, and goat), 3 types of pets (dog, cat, and rabbit), and 3 types of rodents (sewer rat, black rat, and house mouse), for a total of 12 animal types.

Here we introduce examples of analysis of animal types by DNA testing using a combination of the abovementioned method together with Shimadzu's MCE-202 MultiNA microchip electrophoresis system.

■ Experimental Procedure

The DNA extraction method and PCR conditions used were in accordance with the "Animal Hair DNA Testing Protocol"^(*) developed by the Aichi Industrial Technology Institute Food Research Center.

The samples used for PCR consisted of DNA samples of cow, pig, chicken, horse, sheep, goat, dog, cat, rabbit, sewer rat, black rat, and house mouse that were kindly provided by the Aichi Industrial Technology Institute Food Research Center.

In addition to these, DNA was also extracted from the hair of 4 breeds of dog (Shiba, chihuahua, corgi, and Afghan hound), a cat (Norwegian forest cat), and a rabbit (Lionhead rabbit).

Following PCR, the amplified DNA was subjected to size analysis using the MultiNA. The animal types were identified from the analysis results (See procedure flowchart, Fig. 1).

* Animal Hair DNA Testing Protocol
(Aichi Industrial Technology Institute Food Research Center)
<http://www.pref.aichi.jp/cmsfiles/contents/0000016/16149/protocol0821.pdf>

Patent Application Number: Special Application 2007-240023
Invention Name: Animal Identification Primer Set and Primer Kit

The Primer Set is manufactured and marketed by Bex Co., Ltd. with the permission of Aichi Prefecture.
<http://www.bexnet.co.jp>

■ Reagents/Kits

- QIAGEN Fast Cycling PCR Kit (Qiagen) 203743
- Primer Sets for identification of animals (each 12 types) (Bex)
- QIAamp DNA Micro Kit (Qiagen) 56304

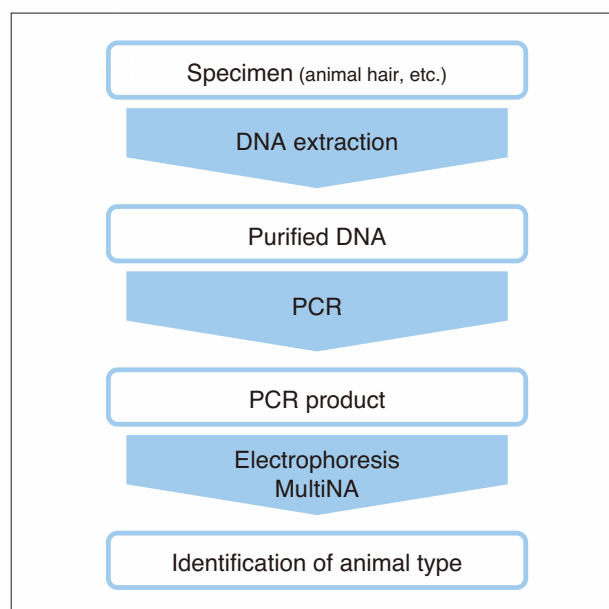


Fig. 1 Experimental Procedure for Identification of Contaminants (Animal Hair) Using DNA Testing

- DNA-500 kit (Shimadzu Corp.) P/N: 292-27910-91
- GelStar[®] Nucleic Acid Stain (Takara Bio) F0535
- 25 bp DNA Ladder (Invitrogen) 10597-011

■ Results

Fig. 2 shows the analysis results for the PCR products of the 12 animal types (cow, pig, chicken, horse, sheep, goat, dog, cat, rabbit, sewer rat, black rat, and house mouse) and dog breeds (Shiba, chihuahua, corgi, and Afghan hound), cat (Norwegian forest cat), and rabbit (Lionhead rabbit).

Characteristic lengths of PCR products (cow (137 bp), pig (230 bp), chicken (159 bp), horse (183 bp), sheep (224 bp), goat (160 bp), rabbit (167 bp), dog (122 bp), cat (220 bp), sewer rat (237 bp), black rat (102 bp), and house mouse (116 bp)) were amplified for the 12 animal types, respectively.

The DNA of the 12 types of animals was detected

from the electrophoresis results of 1 to 12 (Fig. 2). In addition, with the electrophoresis results of a to f (Fig. 2), DNAs of lengths which can be used to identify the animal types were also detected from the respective hair of dog (Shiba, chihuahua, corgi, and Afghan hound), rabbit (Lionhead rabbit), and cat (Norwegian forest cat).

By combining the use of the Primer Sets and the MultiNA microchip electrophoresis system as described in this analysis example, contaminants can be identified from minute quantities of biological substances such as hair, etc., often discovered in manufacturing processes and products.

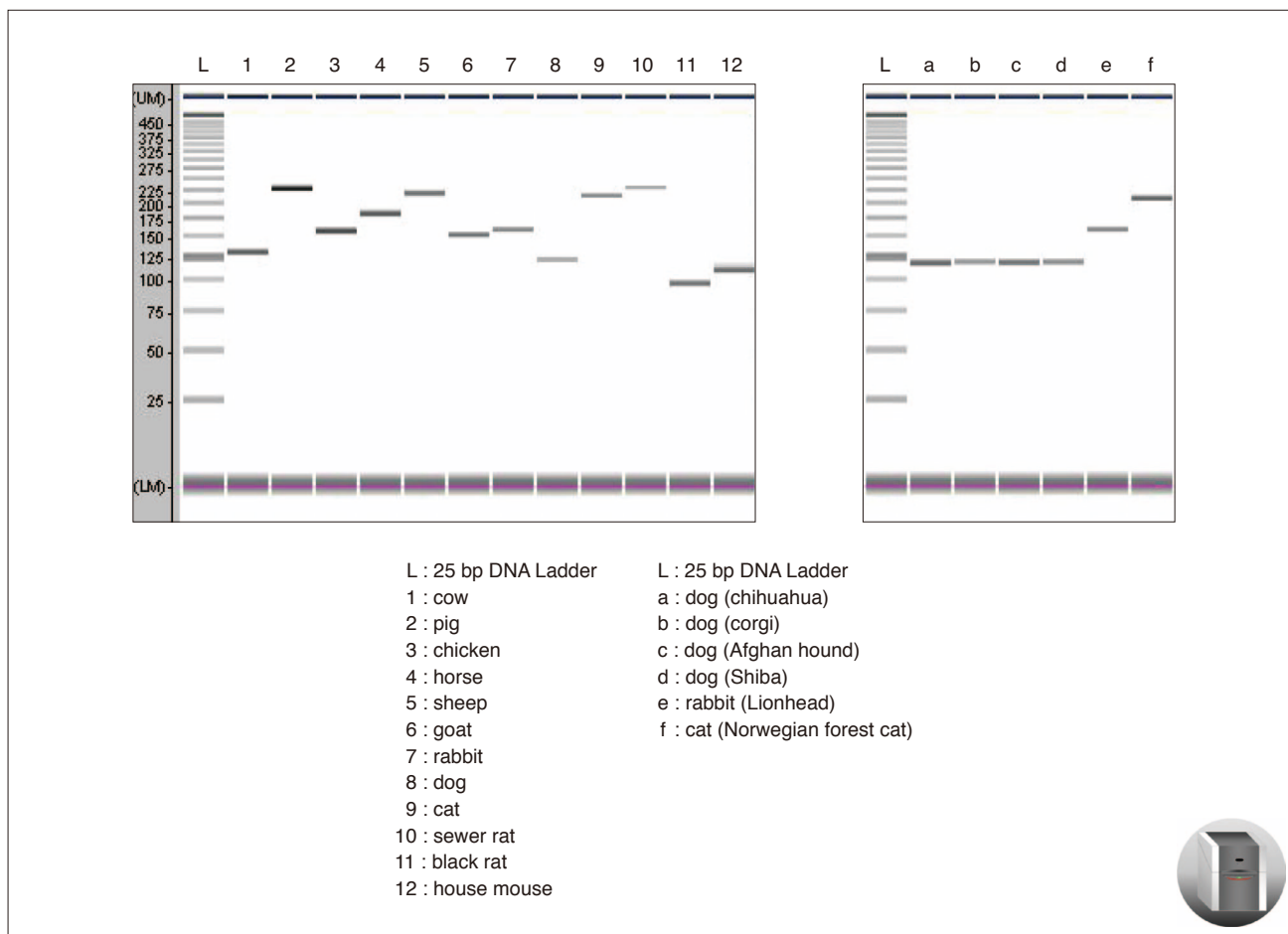


Fig. 2 Analytical Results of PCR Products