3X

APPLICATION OF *PORCINE REPETITIVE ELEMENT* 1 (PRE-1) AS MOLECULAR MARKERS FOR IDENTIFICATION OF SWINE MATERIAL

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ABSTRACT

In order to identify the meat and meat products from different animal species and avoid meat adulteration. This research was conducted by DNA amplification technique of short sequences interspersed nucleotide elements (SINE) for detection of swine material on fresh meat and meat products. Three pairs of primers derived from *Porcine Repetitive Element*-1 (PRE-1), i.e P252 and P642, is used to amplify a sample of DNA containing swine material, which comes from fresh meat and meat products. Isolation of DNA derived from fresh meat or processed meat can produce good quality and quantity of DNA, so it can proceed to DNA amplification by PCR methodSamples of DNA containing swine material can be amplified using primers P252 (495 bp) and P642 (390 bp). Flanking primers PRE-1 has a unique and sensitive primers to detect swine material on fresh meat and meat products. Repetition using PRE-1 primers performed at least three times on each material tested.

Keywords : PRE-1, flanking primers PRE-1, meat and meat product

INTRODUCTION

Indonesian Government validate Law of the Republic of Indonesia Number 8 Year 1999 on Consumers Protection on April 20, 1999. This is a step forward for consumers in Indonesia because of the realization of the hope that this law can protect the public from food that does not meet security requirementsfood both in terms of health, hygiene, nutrition and halal. Along with the enforcement of these laws, various cases still occur related to the problem of "halal" and highly detrimental to consumer especially the Muslim consumer. Halal food is defined as food which does not contain "haram" elements or ingredients or prohibited materials to be consumed by Muslims and its processing does not conflict with Islamic law (Food Act, 2001).

Primers used in this study is a primer that flank PRE-1 sequences by two pairs as shown in Table 1.

Table 1. Primer flanking the PRE-1 sequences

Primer's Name	Base Trace (5'-3')	PCR Product Size (Base Pairs)
P252	F:GCTTACTAACATGAAGGAGCTGTGTAACCTC R: GGCTCACATTCAATCCCTAGCCTTGACCC	495
P642	F:TAACAATGGTTAAGGCGGCAATAGTTATGTG R: TTTAGGTCTACTGTTTGCCAAGTTAGGTGC	390

Sulandari et al. (1997). Description: F = foreward; R = reverse

Isolation and DNA Extraction

Samples of raw and processed products which have been obtained extracted to get the DNA. Isolation and extraction procedure performed according to Sambrook method et al. (1989) which has been modified.

Polymerase Chain Reaction Analysis

Tests done by amplify the DNA sequence contained in samples using PCR. Stages in the PCR analysis of samples are as follows:

- The samples were dissolved in 50 ng aquabidest number included in the PCR special tube.
- Then added the PCR components consisting of buffer solution, dNTP, taq polymerase, primer mix, and H2O (Table 2) and then inserted into a PCR programmed machine as in Table 3.

Table 2. Solution component for PCR reaction

Component	Concentration stock	Concentration required	Volume required (μ l)
Buffer solution	10 x	1 x	1.25
DNTP	2 mg	200 μ g	1.25
Primer mix	400 μ g / μ l	400 μ g	1.00
Taq polymerase	5 units / μ l	1.5 Units	0.30
DNA samples	50 ng / μ l	50 ng	1.00
Water			7.70
Total			12.50

Table 4. The success rate of DNA amplification using the primers P252 and P642

No.	Product Type	P252*					P642*				
		1	2	3	4	Tot	1	2	3	4	Tot
1	Beef lymph	-	-	-	-	0	-	-	-	-	0
2	Beef liver	-	-	-	-	0	-	-	-	-	0
3	Beef heart	-	-	-	-	0	-	-	-	-	0
4	Beef lung	-	-	-	-	0	-	-	-	-	0
5	Beef intestine	-	-	-	-	0	-	-	-	-	0
6	Cowhide	-	-	-	-	0	-	-	-	-	0
7	Beef dried meat	-	-	-	-	0	-	-	-	-	0
8	Shredded beef	-	-	-	-	0	-	-	-	-	0
9	Beef meatballs	-	-	-	-	0	-	-	-	-	0
10	Beef sausage	-	-	-	-	0	-	-	-	-	0
11	Corned beef	-	-	-	-	0	-	-	-	-	0
12	Pork heart	+	+	+	+	4	+	+	+	+	4
13	Pork liver	+	+	+	+	4	+	+	+	+	4
14	Pork lymph	+	+	+	+	4	+	+	+	+	4
15	Pork lung	-	+	+	+	3	+	+	+	+	4
16	Pork intestine	+	-	+	+	3	+	-	+	+	3
17	Pork skin	+	+	+	+	4	+	+	+	+	4
18	Fermented pork sausage	+	+	+	+	4	+	+	+	+	4
19	Pork sausage 1	+	-	+	+	3	-	+	+	+	3
20	Pork sausage 2	+	+	+	+	4	+	+	+	+	4
21	Corned pork 1	+	+	+	+	4	+	+	+	+	4
22	Corned pork 2	+	+	+	+	4	+	+	+	+	4
23	Corned pork 3	-	+	+	+	3	+	-	+	+	3
24	Raw pork dried meat	+	+	+	+	4	+	+	+	+	4
25	Grilled pork dried meat 1	+	+	+	+	4	+	+	+	+	4
26	Grilled pork dried meat 2	+	+	+	+	4	+	+	+	+	4
27	Pork meatballs	+	+	+	+	4	+	+	+	+	4
28	Shredded pork	-	+	+	+	3	+	+	+	+	4

Figure 1 showed the results of PCR using the P642 on various samples of processed meat. PCR products electrophoresis on agarose gel produces long ribbons with

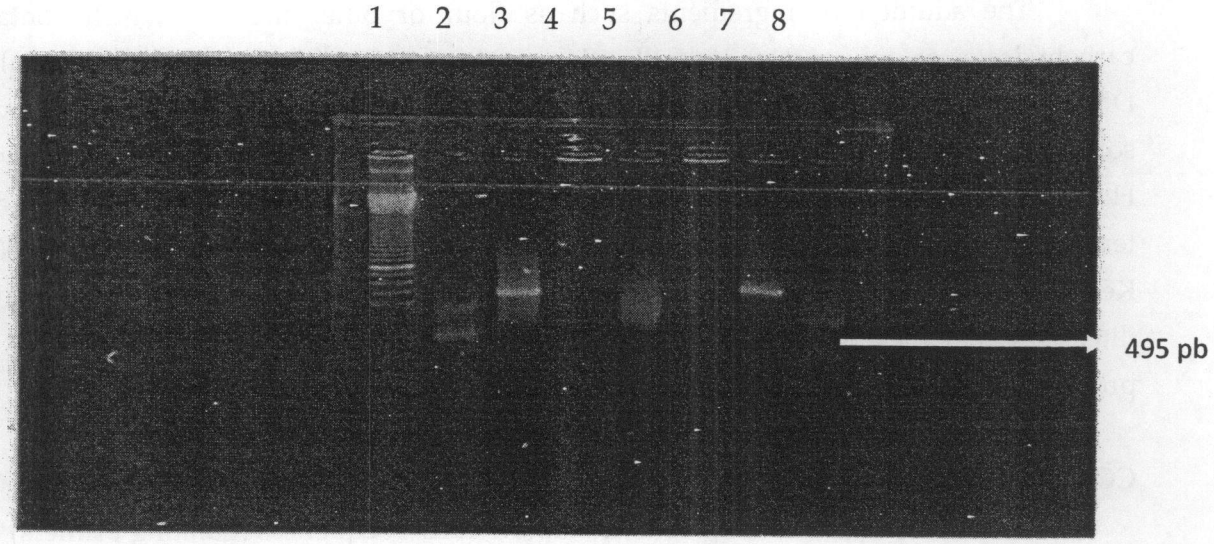

Figure 2. PCR products result using primers P252 in 2% agarose gel. Lanes: (1) Marker 100 bp, (2) Corned 2; (3) Corned 1; (4) Corned 1; (5) Corned 2; (6) Sausage 1; (7) Shredded1; (8) Shredded 2.

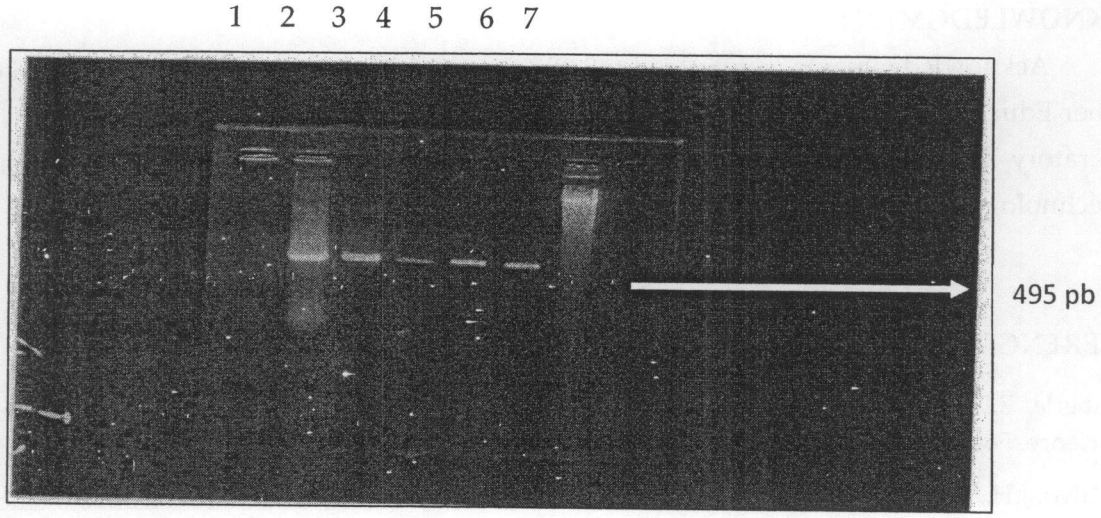

Figure 3. PCR products result using primers P252 in 2% agarose gel. Lanes: (1) Meatball 1; (2) Meatballs, (3) Shredded, (4) raw dried meat; (5) Grilled minced dried meat (6) Grilled dried meat; (7) Marker 50 bp.

Meat processing techniques in general will not damage DNA molecules in the tissues. This can be seen from the level of success when perform DNA isolation from processed meat products which are treated in various ways. Shredded, corned beef and sausage are some examples of products that have higher difficulty level than other products (dried meat and meat balls) at extraction and isolation step of DNA. This situation is understandable because these three products contain more diverse and less meat components than others.

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