A PRACTICAL APPLICATION OF DNA PROBES IN QUALITY ASSURANCE OF MEAT PRODUCTS - THE SEX TEST

Klaus I, *MATTHAEI*, Ken* C. *REED*, Terry* J. *NICHOLLS*** and *Ian B. STEPHENS****

SUMMARY

An assay developed by A.B. Technology for determining the sex of livestock embryos has been adapted to compliance testing for the premium bull meat trade. Analysis of product quality is now routine *in* an area that previously has been impossible to monitor. The assay is based on specific detection of Y-chromosomal DNA, and is the first example of what is anticipated to be a broad range of applications of DNA probes in the industry.

INTRODUCTION

The export meat trade is highly competitive, subject to the vagaries of nature, international politics and the global exchange rate. It is hardly surprising that exporters have been tempted to substitute frozen packs of beef with cheaper meat from other species. Introduction of the Checkmeat system, based on random sampling and assay of bonded product with an eight species enzyme-linked immuno-absorbent assay, has virtually eliminated species substitution. Nevertheless, some aspects of quality assurance testing remain difficult to test objectively, age and sex being two examples. Bull meat is a premium product because of its water retention qualities which make it an ideal base for the manufacture of sausages and related smallgoods. Since relatively few bulls are available for slaughter, the probability is high that meat from culled dairy cows may be substituted. There has been no possibility of detecting this type of substitution after the meat has been boned out and frozen.

A.B. Technology Pty. Limited has implemented a unique assay to determine the genetic sex of bovine embryos, based on the use of a DNA probe which detects Y-chromosomal (male-specific) DNA (Reed *et al.* 1988). In consultation with the DPIE Compliance Branch and Bureau of Rural Resources, this assay has been modified to differentiate the genetic sex of samples taken from packs of frozen cattle meat. The method simultaneously tests for species of origin, and can be applied to sex analysis of tissue samples from other domestic livestock species.

MATERIALS AND METHODS

Preparation of meat samples for analysis

Core samples of tissue were removed from opened cases of frozen meat with a sterilised *twist* drill after surface tissue had been removed with a sterile chisel. Approximately 1 mg of each sample was suspended in 0.3 ml of 0.4 M NaOH, 10 mM EDTA (SHE) in a small sealed tube and digested by heating at 95°C for 20 min. A series of two-fold serial dilutions (0.25 ml) was prepared in a 96-well microtitre plate for each digested sample ("B" dilution series). Five microlitre aliquots of each dilution were then removed into 0.2 ml of SHE in a second 96-well plate ("A" dilution series).

The entire contents of every well in both plates were filtered onto Zeta-Probe (Bio-Rad) nylon membrane (Chomczynski and Qasba 1984; Reed 1990) using a 96-

* *A.B. Technology Pty. Limited, c/- Dept Biochemistry, Faculties, Australian National University, Canberra, A.C.T. 2601.*
** *Bureau of Rural Resources, DPIE, John Curtin House, Barton, A.C.T. 2600.*
*** *Bureau of Rural Resources, DPIE, Brisbane, Qld. 4000.*

well dot blot filtration manifold (Bio-Rad). In this way 96% of each dilution was transferred onto one membrane ("B"; for hybridisation with pBCY11a) and 4% of each dilution was transferred onto a second membrane ("A"; for hybridisation with pBKS). Samples of serially diluted DNA from male and female cattle were simultaneously filtered onto each membrane to provide positive and negative controls. After filtration the membranes were neutralised by rinsing in 5 x SSC (0.75M NaCl, 0.075M trisodium citrate, pH 7) and washed in 5 x SSC, 1% (w/v) SDS (sodium dodecyl sulphate) at 68°C for 2 h.

DNA probes

The Y-chromosomal probe (pBCY11a) was a plasmid containing a cloned insert of bovine cDNA that is transcribed in the testis and is repeated several hundred-fold in the genomic DNA of male, but not female, bovid ruminants (Reed *et al.* 1988). The autosomal probe (pBKS) was a plasmid containing a cloned insert of bovine autosomal satellite DNA (satellite I; Taparowsky and Gerbi 1982; Matthaei and Reed 1986; Reed *et al.* 1988) that is repeated approximately 100,000-fold in the genomic DNA of both male and female cattle. Both probes were radioactively labeled with [alpha-^{32}P] dCTP by nick translation (Rigby *et al.* 1977; Reed 1990) to a specific activity of approximately 2 x 10^8 dpm/ug.

DNA hybridisation analysis

The membranes were incubated in sealed plastic bags with radioactively labeled probe DNA (pBCY11a with membrane "B"; pBKS with membrane "A") in a solution of 0.267 M sodium phosphate, pH 6.9, 2 mM EDTA, 7% (w/v) SDS, 1% (w/v) BSA (bovine serum albumin) at 68°C for 14 h, washed free of excess probe solution (Church and Gilbert 1984; Reed 1990), then exposed to Fuji RX X-ray film at room temperature for 2 h (pBKS) or to Kodak XAR film at -70° for 12 h with a DuPont Cronex "Lightning Plus" intensifying screen (pBCY11a).

RESULTS

The principles and selectivity of the assay are illustrated by the data of Fig. 1. Figure lb demonstrates clearly the discrimination between male and female bovine DNA and meat samples provided by the Y-chromosomal probe. The similar reactivity of samples from both sexes with the autosomal probe pBKS (Fig. la) provides an unambiguous baseline for the Y-analysis. The specificity of the autosomal probe for bovine DNA is illustrated in Fig. la by its lack of reactivity with pig samples, but has been demonstrated more extensively for a number of other species (data not included). The male-specific probe pBCY11a reacts similarly with tissue samples of domestic cattle, sheep and goats and a number of wild ruminant species (data not included).

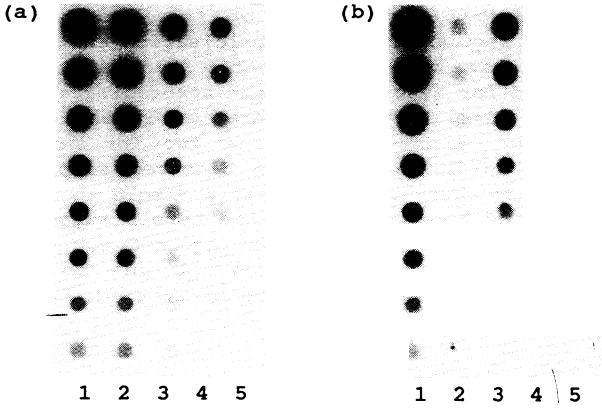

Fig. 1. Hybridisation of DNA and meat samples of known origin with autosomal probe pBKS (a) and Y-chromosomal probe pBCY11a (b). Each vertical column of dots is a series of doubling dilutions, where the first dot contains 0.04 ug (a) or 1 ug (b) of DNA (1. male cattle; 2. female cattle) or approx. 0.017 mg (a) or 0.4 mg (b) of meat (3. male cattle; 4. female cattle; 5. male pig).

Application of the assay to *frozen* meat samples is shown by the example of Fig. 2. DNA in the samples was quantified and shown to be bovine by hybridisation with pBKS (Fig. 2a). The genetic sex of the samples is strikingly obvious from their reactivity with the Y-chromosomal probe (Fig. 2b), which reveals that three of the samples on this pair of membranes were taken from male (nos. 2, 5, 7) and five from female meat (nos. 1, 3, 4, 6, 8).

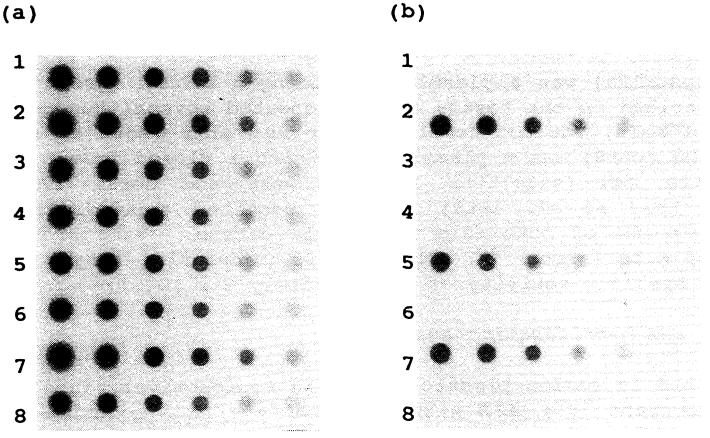

Fig. 2. Hybridisation of eight unknown meat samples with autosomal probe pBKS (a) and Y-chromosomal probe pBCY11a (b). Each horizontal row of dots is a series of doubling dilutions where the first dot contains approx. 0.017 mg (a) or 0.4 mg (b) of meat.

DISCUSSION

Hybridisation of tissue samples with DNA probes provides a rapid, sensitive, definitive and economical test for the presence of defined genetic traits. In this paper we present a simple method for applying this technique to the determination of genetic sex of meat samples. The test has the added advantage of simultaneously confirming the species of origin. Although relevant data are not presented in this paper, similar tests can be applied with equal facility to sheep, goat, deer and pig meat samples using currently available probes for species'-specific autosomal satellite DNA and for Y-chromosomal DNA sequences. The assay's utility could be further extended to monitoring for the presence of sex hormones as exogenous growth promoting substances by parallel application of our test for genetic sex with assays for sex hormones.

This is the first such application of its type, providing the Australian industry with a unique ability to improve surveillance of the quality of certified meat products. The resources required for DNA hybridisation analysis are independent of sample number and are commonly available in analytical laboratories; while two days is required for hybridisation and autoradiography, this is instrument time, not personnel time. Sample preparation is the only labour-intensive component of the assay, requiring a day or two for manual preparation of six dilutions for each of 120 weighed and digested samples. However, the basic sample preparation procedure is sufficiently simple to lend itself to automation, while validation *of* the assay described herein suggests that in routine application no more than two replicates are required for each sample. The simplicity of the assay allows its application not only to the unequivocal resolution of suspect consignments but to routine monitoring.

REFERENCES

CHOMCZYNSKI, P. and QASBA, P.K. (1984). Biochem. Biophys. Res. Commun. 122: 340.
CHURCH, G.M. and GILBERT, W. (1984). Proc. Natl. Acad. Sci. USA 81: 1991.
MATTHAEI, K.I. and REED, K.C. (1986). Promega Notes 5: 5 (Promega: Madison).
REED, K.C. (1990). In "Methods in Gene Technology", Vol. 1, in press, editors J.W. Dale and P.G. Sanders. (JAI Press: London).
REED, K.C., LORD, E.A., MATTHAEI, K.I., MANN, D.A., BEATON, S., HERR, C.M. and MATTHEWS, M.E. (1988). Patent application PCT/AU89/00029.
RIGBY, P.W.J., DIECKMANN, M., RHODES, C. and BERG, P. (1977). J. Mol. Biol. 113: 237.
TAPAROWSKY, E.J. and GERBI, S.A. (1982). Nucleic Acids Res. 10: 1271.