Suitability Of Bovine Sexing Primers For Sex Determination Of Caprine Cells

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ABSTRACT

Our study investigated the suitability of widely used bovine sexing primers for their usefulness in sexing caprine cells. The common use of these sexing primers is to amplify BRY.4, a Y-specific sequence found in bovine cells (Reed, 1995). The major advantage in amplifying these sequences is that they are multiple repeated DNA sequences. This enhances the sensitivity for use on as few as one cell. We digested the DNA with Hinf1 either prior or subsequent to PCR amplification in order to eliminate a homologous repeat sequence found in female cells. Our study investigated whether a site homologous to the Y-specific sequence was also present in female caprine cells and, if so, it too contained the Hinf1 restriction site, making possible the elimination of the troublesome sequence. Lymphocytes from one male and three female goat species were used as the source of DNA. Approximately 100 cells/2 µL were used to ensure the amplification of the sequence homologous to the Y-repeat. After replication, some samples were digested with Hinf1. The DNA from three female undigested samples banded at the same position as the male samples. The DNA from all three female digested samples banded at two positions. The male DNA remained unaltered after the Hinf1 digest. The assay generated strong sexing bands with as few as 6 male lymphocytes, the lowest number of cells tested.

INTRODUCTION

Recent studies on PCR-based sex determination of caprine cells use amplification of a single-copy Y-chromosome-specific sequence. Our study investigated the suitability of widely used bovine sexing primers for their usefulness in sexing caprine cells. The common use of these sexing primers is to amplify BRY.4, a Y-specific sequence found in bovine cells (Reed et al., 1995). The major advantage in amplifying these sequences is that they are multiple repeated DNA sequences. This enhances the sensitivity for use on as few as one cell. Early in their use, a problem was discovered amplifying BRY.4 for bovine sexing: one or more copies of a homologous repeat sequence were present in female cells. This was also verified by a dot blot analysis. Our sequence analysis

INTRODUCTION continued

in bovine revealed that copies in female cells differed by the presence of a Hinf1 restriction site. By digesting the DNA with Hinf1 either prior or subsequent to PCR amplification, it was possible to eliminate or identify the troublesome sequence, respectively. Our study investigated whether a site homologous to the Y-specific sequence was also present in female caprine cells and, if so, it too contained the Hinf1 restriction site, making possible the elimination or identification of the troublesome sequence.

MATERIALS AND METHODS

DNA was isolated from one breed of male and three breeds of female goats: Saanen, Nubian/Alpine mix, and Albino. Lymphocytes were allocated into concentrations of 6, 20, and 100 cells per 2 µL. The components of the PCR reaction mix were: buffer (50 mM Tris, 1% dextran T-500, 50 mM KCl, 2.5 mM MgCl2, and 0.035% 2-mercaptoethanol), deoxyribonucleotides (5 µM) (Boehringer Mannheim, Basel, Switzerland), primers (5′-GAACTTTCAAGCAGCTGAGGC-3′ and 5′-GATTGTTGATCCCACAGAAGG-3′) (2.5 µM) (custom synthesis, IDT Inc, Coralville, IA, USA)), and Taq polymerase (2.7U)(AmpliTaq DNA Polymerase, Stoeffel Fragment, Perkin Elmer, Branchburg, NJ, USA). Approximately 100 cells/2 µL were used to ensure the amplification of the sequence homologous to the Y-repeat. The DNA replication was carried out using a Corbett Rapid Thermocycler (Model FTS-IS, Corbett Research, Montlake, Australia) in 20-µL volumes, and all assays were run with positive and negative DNA controls. After replication, some samples were digested with Hinf1 (20 U) (New England Biolabs, Ipswich, MA, USA) for 2 h at 38°C. A 3% agarose gel was run in an agarose gel electrophoresis apparatus (Model 52000, IBI-Shelton Scientific, Peosta, IA, USA) for 30 min at 180 volts. All caprine species samples were run in duplicate.

RESULTS

The DNA from three female undigested samples banded at the same position as the male samples (130 bp). The DNA from all three female digested samples banded at two positions (50 bp and 80 bp), both lower than the male samples. The male DNA remained unaltered after the Hinf1 digest. The assay generated strong sexing bands with as few as 6 male lymphocytes, the lowest number of cells tested.

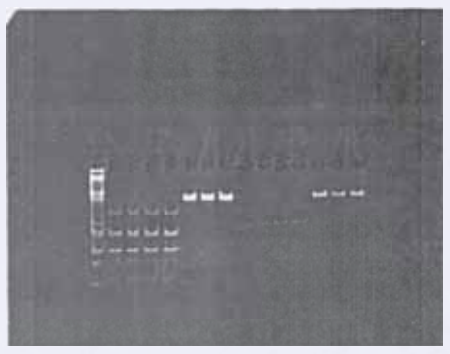

Figure 1: A representative gel. From left to right: Ladder, female samples cut with Hinf1 after PCR, female uncut samples, female samples cut before PCR, female uncut samples.

DISCUSSION

The male DNA remained unaltered after the Hinf1 digest, which suggests that the Hinf1 site is not present in the amplified DNA from male cells. These primers seem suitable for sexing in caprine cells if used in conjunction with Hinf1 digestion.

REFERENCES

Reed *et al*. 1995 U.S. Pat# 5459038

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