Materials and methods

DNA extraction from rumen fluid

Briefly, 0.5 ml of frozen rumen fluid was thawed on ice before transferring to a 2 ml screw cap tube containing zirconia beads (0.3 g of 0.1 mm and 0.1 g of 0.5 mm diameter). The cells were lysed by homogenization in a Fast Prep® tissue and cell homogenizer at 6 ms^{-1} for 3 min, incubated at 70˚C for 15 min and finally centrifuged at 10˚C at 17, 900 x *g* for 5 min. The supernatant from each sample was then transferred to a new 1.5 ml sterile screw cap tube and protein was removed by precipitation with 420 µl of 10 M ammonium acetate. DNA was precipitated by addition of 700 µl isopropanol to the supernatant from the previous step and incubated at -20°C overnight. After centrifugation (17,900 x *g* at 4˚C for 15 min), the DNA pellet was washed with 750 µl of 70% (v/v) ethanol, then resuspended in 100 µl of TE (10 mM Tris Cl pH 8.0, 1mM EDTA). RNA was removed from DNA samples by treating with DNase-free RNase (Promega UK Ltd., Southampton, UK). DNA was then purified using the QIA amp DNA Stool Mini Kit (Qiagen Ltd, Crawley, UK) as previously described (Yu and Morrison, 2004). The integrity of total microbial high molecular weight DNA and successful removal of RNA were verified by agarose gel electrophoresis. The concentration and quality of DNA was determined at A_{260} nm and A_{280} nm with a NanoDrop® ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA).

Yu, Z., and Morrison, M. (2004) Improved extraction of PCR-quality community DNA from digesta and fecal samples. *Biotechniques* **36**: 808-812.