Volume 16 Number 3 1988 Nucleic Acids Research

A simple and efficient method for isolating high molecular weight DNA from mammalian sperm

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Submitted December 16, 1987

We needed DNA from pigs to complete a pedigree using RFLP analysis, however the animals were not present for sampling. Stored semen was available but isolating DNA from semen proved a formidable task. Procedures (1, 2) that included different combinations of high NaCl concentrations, protease K digestion, 1% SDS, 2% 2-mercaptoethanol, ether-alcohol and phenol-chloroform extraction failed to give adequate yields of DNA. Microscopic examination revealed that sperm cells were not completely lysed. We modified a method (3) frequently used for the isolation of RNA, and found that sperm cells readily lysed in guanidinium thiocyanate without homogenization or sonication and resulted in a good yield of DNA.

Centrifuge 1 ml of semen in a microfuge for 5 min.. Wash the pelleted sperm twice in 0.15 M NaCl, 2 mM EDTA. Add 5 ml of 6 M guanidinium thiocyanate, 25 mM Na citrate, pH 7.0, 0.5% Sarkosyl, 0.1 M 2-mercaptoethanol and incubate for 30 min., 37°C. Dissolve 1 gm of CsCl per 2.5 ml solution and layer on 3 ml of 5.7 M CsCl, 0.1 M EDTA, pH 7.0. Centrifuge for 20 hrs., 20°C at 30,000 rpm in a SW41Ti rotor. Remove the upper protein layer. Save the banded DNA which is near the interface of the guanidinium thiocyanate and CsCl. Dialyse for 24 hrs. against 10 mM Tris-HCl, pH 8.0, 1 mM EDTA (TE buffer). Precipitate the DNA with 2 volumes ethanol and wash twice in 70% ethanol. Dissolve the DNA in TE buffer.

The important component of the technique is the ability of the guanidinium thiocyanate to completely lyse the sperm. We suspect that other strong chaotropic agents such as guanidine HCl may be used as well as alternative methods of retreiving the DNA from the mixture. The yield of DNA is $\lesssim$ 200 ug/1x10^8 spermatozoa. The DNA at this point can be used for restriction enzyme digestion (Fig. 1).

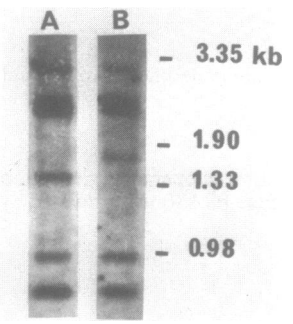

Fig. 1. Southern blot analysis of DNA from porcine sperm (A) and endothelial cells (B). Ten ug of each DNA were digested with the restriction enzyme Taq I and probed with a partial human vWF cDNA (pvWFIPC8). Molecular weight markers are indicated on the right.

BRB is a recipient of grants from the Philippe Foundation. New York/Paris and Fondation pour la Recherche Médicale Française, Paris.

References. (1) Borenfreund et al. (1961) Nature 191, 1375. (2) Bethesda Research Lab (1984) Focus 6:2, 9. (3) Chirgwin et al. (1979) Biochemistry 18, 5294.

