Methodology Report

Genomic DNA Extraction from Sperm

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Received: 26.08.2015
Revised: 16.09.2015
Accepted: 13.11.2015

Abstract

Unlike the somatic cells, sperm DNA is very compact due to replacement of histones with protamines. Disulfide bridges formed within and between the protamines inhibit the extraction of sperm DNA through standard techniques used for the somatic cells. Furthermore, the spermatozoa themselves are protected by a membrane which is rich in disulfide bonds, making cell lysis very difficult. Following a comprehensive literature search, we developed a protocol for DNA extraction from sperm and semen fluid. The quality of extracted DNA was checked running on agarose gel, used for bisulfite conversion and PCR amplification.

Keywords: Sperm, Disulfide bonds, Chromatin, Histones, Protamines

https://doi.org/10.18869/nrip.jamsat.1.2.120

Introduction

During spermatogenesis, replacement of 90-95% of histones with protamines (protamination) makes the sperm chromatin structure very dense. Protamination of sperm chromatin allows the nuclear compaction necessary for sperm motility and helps to protect the genome from oxidation and harmful molecules within the female reproductive tract (1). Compacted chromatin due to disulfide bridges within and between protamines as well as the presence of disulfide bonds in the outer membrane of the sperm make sperm cells resistant to conventional lysis procedures for DNA extraction (2).

Among commercial kits, only QIAGEN offers a procedure for DNA extraction from sperm (customer-adapted protocol) through combining two other kit protocols. Like other many commercially available protocols this one is limited for routine use in laboratory due to high cost.

Conducting a literature search, we found few studies which introduce methods for DNA extraction from sperm. Use of guanidine thiocyanate as protein denaturant was first introduced by Bahnak and colleagues to extract porcine sperm DNA (3). Later, other researchers added further components to the protocol such as proteinase K to increase the activity of guanidine thiocyanate and more efficient digestion of the nucleoproteins or DTT (dithiothreitol) a reducing agent, which cleaves disulfide bonds and allows proteins to unfold (4-6). Other researchers used bead-based homogenization to facilitate sperm cell lysis in combination with commercially available kits and substituted DTT by Tris (2-carboxyethyl) phosphine (TCEP), an odorless reducing agent which is found to be more stable and more powerful than the commonly used reducing agents (7).

Our reviewed literature revolved around justifying this argument (3,4,11). By testing all lysis buffer recipes, and subsequent methods for protein removals and DNA precipitation from lysate, we proposed the present protocol. The integrity of extracted DNA was examined running on agarose gel confirming favorable yield and quality of DNA.

The extracted DNA was used in our further experiments using bisulfite conversion and PCR amplification leading to promising results.

Materials

The following materials were employed upon the development of the present protocols: Lysis buffer (10 mM TRIS HCl (pH 8.0), 25 mM EDTA, 1% SDS, 75 mM NaCl), Triton-X100, Dithiothreitol (DDT), Proteinase K (20 mg/ml), 3M Sodium acetate (NaAc), Phenol/chloroform/isoamyl (25:24:1) (PH: 8), Glycogen, Ethanol 100% and 70%.

Procedure

Based on the available evidence and our pilot experiments, the below recipe has been proposed:

1) Centrifuge semen/sperm to achieve cell pellet.

2) Add 500 µl lysis buffer to cell pellet + 2.5 µl Triton X-100 + 21 µl DDT + 10 µl P.K.

3) Mix well and keep overnight at 50 C° (agitation during incubation is recommended).

4) Centrifuge in high velocity to put down all debris and continue to extract the supernatant by phenol/chloroform/isoamyl (25:24:1, PH: 8) method.

5) Add the same supernatant volume (approximately 400 µl) phenol/chloroform/isoamyl.

6) Mix well and centrifuge at high velocity for 5 minutes to separate DNA from proteins.

7) Take the upper aqueous phase (DNA), without disturbing the middle phase and transfer to new 2 ml eppendorf tube. Proceed DNA precipitation by adding 1/10 volume NaAc (3M, PH: 5.2) + 1 µl Glycogen + 2 volumes Ethanol 100% (respect this order for adding above materials). Mix well and incubate 1 hour at -80 C°.

8) Centrifuge at high velocity in 4 C° for 15 minutes to precipitate DNA.

9) Throw the alcohol and wash DNA pellet by cold ethanol 70%, centrifuge at high velocity for 5 minutes.

10) Aspirate the ethanol completely without disturbing the pellet and air dry for approximately 15-20 minutes and then resuspend DNA in 50-100 µl ddH20 or TE buffer.

Results and quality control

The integrity of DNA was checked by running on agarose gel. As illustrated in figure1, 500 ng and 1000 ng of the random sample were loaded on 1.5% agarose gel. The presence of sharp bands indicate an integrated DNA.

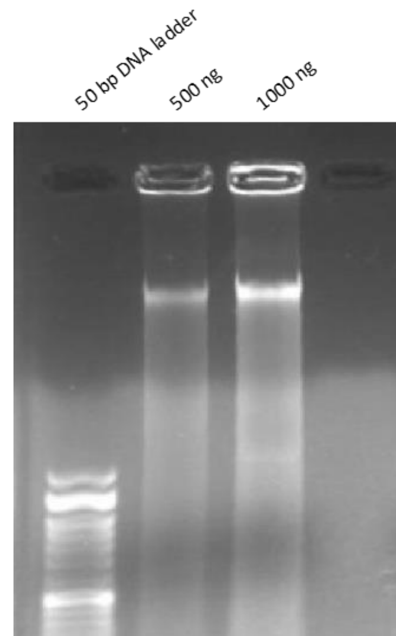

Figure1: DNA quality control on agarose gel.

Acknowledgment

Authors wish to thank Alexandra Weyrich and Claudia Gebert for their continuous technical and scientific support and described protocols for genomic DNA extraction from sperm.

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