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DNA isolation from cattle semen for long read sequencing

 PLOS One Peer-reviewed method

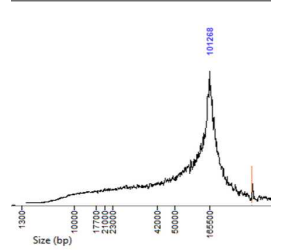
DOI

<https://dx.doi.org/10.17504/protocols.io.j8nlkw1qwl5r/v1>Erwan Denis¹, Cecile CG Grohs², Carole Iampietro¹¹INRAE, US 1426, GeT-PlaGe, Genotoul, France Genomique, Université Fédérale de Toulouse, Castanet-Tolosan, France;²Université Paris-Saclay, INRAE, AgroParisTech, GABI, 78350, Jouy-en-Josas, France

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Protocol status: Working

We use this protocol and it's working

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Abstract

Here we describe a method for isolate high molecular weight DNA from commercially available frozen bull semen straws.

This protocol is based on a salting-out method and uses several commercially available solutions. It consists of several steps: washing of semen, lysis, removal of proteins and precipitation of genomic DNA.

This protocol was used to isolate DNA from sixty semen straws, all of which were successfully sequenced using the CLR sequencing mode on the PacBio SequelII platform.

Guidelines

Salting out is a good method to obtain high molecular weight (HMW) DNA, as it avoids damaging steps such as the use of purification columns or heavy mixing with phenol/chloroform.

Note that all mixing steps should be gentle to obtain HMW DNA fragments (from lysis steps to DNA precipitation).

We also recommend to use DNA low bind tubes.



Protocol materials



Tris(2-carboxyethyl)phosphine hydrochloride solution **Merck MilliporeSigma (Sigma-Aldrich) Catalog #646547-10X1ML**



Buffer RLT **Qiagen Catalog #79216**



Phosphate-buffered saline, pH 7.4



DNA LoBind Tubes 2.0 mL **Eppendorf Catalog #30108078**



EB buffer **Qiagen Catalog #19086**



Isopropanol



Puregene Tissue Kit **Qiagen Catalog #158063**



Proteinase K **Qiagen Catalog #19133**

Safety warnings



See Safety Data Sheets for warnings and safety hazards.

Before start

As we use commercial sperm straws to perform our extractions, we do not always know the composition of these straws, the quantity of material contained, the nature of the diluents and preservatives used. This is why it is sometimes necessary to use several straws to obtain enough material for sequencing. It is also sometimes wise to perform several washes (see step 3) to eliminate contaminants from diluents and preservatives.



Preparation of reagents

- 1 Immediately before use, prepare a mix containing RLT buffer (Qiagen) and TCEP [Tris(2-carboxyethyl)phosphine hydrochloride] to a final volume of 500µL per sample as follow:

-  450 µL RLT

-  50 µL TCEP

 Buffer RLT **Qiagen Catalog #79216**

 Tris(2-carboxyethyl)phosphine hydrochloride solution **Merck MilliporeSigma (Sigma-Aldrich) Catalog #646547-10X1ML**

Note

This mixture of a guanidine-based reagent (RLT) and a thiol-free reducing agent facilitate dissociation of disulfide bonds (Wu *et al*, 2018).
TCEP is odorless, and more stable than DTT (Han & Han, 1994).

Citation

Han JC & Han GY (1994)

. A Procedure for Quantitative Determination of Tris(2-Carboxyethyl)phosphine, an Odorless Reducing Agent More Stable and Effective Than Dithiothreitol.
Analytical Biochemistry.

<https://doi.org/10.1006/abio.1994.1290>

LINK

Citation

Wu H, de Gannes MK, Luchetti G, Pilsner JR (2015)

. Rapid method for the isolation of mammalian sperm DNA.

<https://doi.org/10.2144/000114280>

LINK



Preparation of sample

2 Recovery of spermatozoa from the straw:

- Empty the  200 μ L  Sample in a  2 mL tube by cutting the two ends of the straw

 DNA LoBind Tubes 2.0 mL Eppendorf Catalog #30108078

- Rinse the straw it with  200 μ L 1X PBS  Room temperature

 Phosphate-buffered saline, pH 7.4

3 Wash:

10m

- Add  800 μ L more PBS (up to  1 mL 1X PBS)
- Pellet  1000 x g, Room temperature, 00:05:00
- Discard the supernatant

Second wash is optional (no significant impact observed)

- Re-suspend in  1 mL 1X PBS
- Pellet  1000 x g, Room temperature, 00:05:00
- Discard the supernatant

Note

Centrifuge gently so that the pellet does not stick. It should be easy to resuspend for efficient lysis.

Lysis

4 Step one:

10m 10s

- Add  500 μ L of RLT-TCEP to the pellet



- Vortex  00:00:10 by pulsing at max speed
- If necessary, use a wide opening tip to resuspend the pellet
- Incubate  On ice  00:10:00

5 **Step two:** continue with Qiagen Puregene Tissue kit adapted as follow

1h 30m

- Add  500 μL of Cell Lysis Solution
- Add  60 μL of  20 mg/mL proteinase K (20 mg/ml)
- Mix by inversion (about 25 inversions)
- Incubate  55 °C  01:30:00

 Puregene Tissue Kit **Qiagen Catalog #158063** Proteinase K **Qiagen Catalog #19133**

6 **Remove RNA:**

16m

-  3 μL RNase from Qiagen Puregene Tissue Kit
- Incubate  37 °C  00:15:00
- Incubate  On ice  00:01:00

Protein precipitation

1m 15s

- ### 7
- Add  200 μL of Protein precipitation buffer (from Qiagen Puregene Tissue Kit)
 - Mix by hand or gently vortexing  00:00:15
 - Incubate  On ice  00:05:00
 - Centrifuge  16000 x g, Room temperature, 00:01:00

6m 15s

DNA precipitation

6m

- ### 8
- Transfert the supernatant to a new tube containing  600 μL of Isopropanol
 - Carrefully invert the tube 25-50X times to form the pellet
 - Incubate  00:05:00  Room temperature
 - Centrifuge  16000 x g, 00:01:00
 - Discard supernatant

 Isopropanol



- 9
- Add  600 μL of 70% ethanol to the pellet
 - Centrifuge  5000 x g, 00:02:00
 - Discard supernatant
 - Almost dry the pellet  Room temperature  00:05:00
 - Add  50 μL to  100 μL of EB (Qiagen) or TE buffer to eluate DNA
 - Store DNA at 4°C

 EB buffer **Qiagen Catalog #19086**

Note

DNA in EB buffer can be heated to 60°C for 1 hour to dissolve it. Do not vortex or pipet DNA.
It is recommended not to freeze the DNA to preserve long fragments.

Expected result

Of the 60 extractions carried out using this protocol, the average size of the fragments generated is around 53 kb, ranging from 25 to 120 kb on average. We expect 30 μg of DNA from a commercial semen straw, but this figure can vary considerably from sample to sample. We obtained absorbance ratios of 260/280 for DNA of around 1.8 nm, and 260/230 ratios averaging 0.5 nm. Low ratios have already been observed using RLT buffer (Wu *et al*, 2018), but these did not affect PacBio sequencing significantly. Some of these DNA have been sequenced and published in Jourdain *et al*. 2023.

Citation

Jourdain J, Barasc H, Faraut T, Calgaro A, Bonnet N, Marcuzzo C, Suin A, Barbat A, Hozé C, Besnard F, Taussat S, Grohs C, Kuchly C, Iampietro C, Donnadieu C, Pinton A, Boichard D, Capitan A (2023)

. Large-scale detection and characterization of interchromosomal rearrangements in normozoospermic bulls using massive genotype and phenotype data sets.

<https://doi.org/10.1101/gr.277787.123>

LINK



Citations

Step 1

Han JC & Han GY. A Procedure for Quantitative Determination of Tris(2-Carboxyethyl)phosphine, an Odorless Reducing Agent More Stable and Effective Than Dithiothreitol
<https://doi.org/10.1006/abio.1994.1290>

Step 1

Wu H, de Gannes MK, Luchetti G, Pilsner JR. Rapid method for the isolation of mammalian sperm DNA.
<https://doi.org/10.2144/000114280>

Step 9

Jourdain J, Barasc H, Faraut T, Calgaro A, Bonnet N, Marcuzzo C, Suin A, Barbat A, Hozé C, Besnard F, Taussat S, Grohs C, Kuchly C, Iampietro C, Donnadieu C, Pinton A, Boichard D, Capitan A. Large-scale detection and characterization of interchromosomal rearrangements in normozoospermic bulls using massive genotype and phenotype data sets.
<https://doi.org/10.1101/gr.277787.123>