Introduction

This protocol describes a method to extract high molecular weight genomic DNA from bull (*Bos taurus*) sperm, as an example of mammalian sperm. The sample was split into different fractions, lysed separately with guanidine hydrochloride (adapted from the Macherey-Nagel NucleoSpin Tissue protocol), then pooled together and purified with the QIAGEN Genomic-tip 100/G. Sequencing performance was assessed using the MinION. Prior to library preparation, the extracted DNA was sheared using either the Covaris g-TUBE™ or the Diagenode Megaruptor® 3 to increase flow cell output from the sequencing libraries.

Materials

- Bull sperm straw
- QIAGEN Blood and Cell Culture DNA Midi Kit
- QIAGEN ATL buffer
- Guanidine hydrochloride
- Proteinase K
- Tris-HCl, pH 8.0
- 5 M NaCl
- 0.5 M EDTA
- 1 M NaOH
- β-mercaptoethanol
- TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0)
- Isopropanol
- 70% ethanol
- Vortex mixer
- 1.5 ml Eppendorf DNA LoBind tubes
- 15 ml and 50 ml Falcon tubes
- Ice bucket with ice
- Refrigerated microcentrifuge
- Refrigerated centrifuge with capacity for 15 ml and 50 ml Falcon tubes
- Incubator or water bath with capacity for 37°C and 60°C
- Optional: Covaris g-TUBE™ or Diagenode Megaruptor® 3

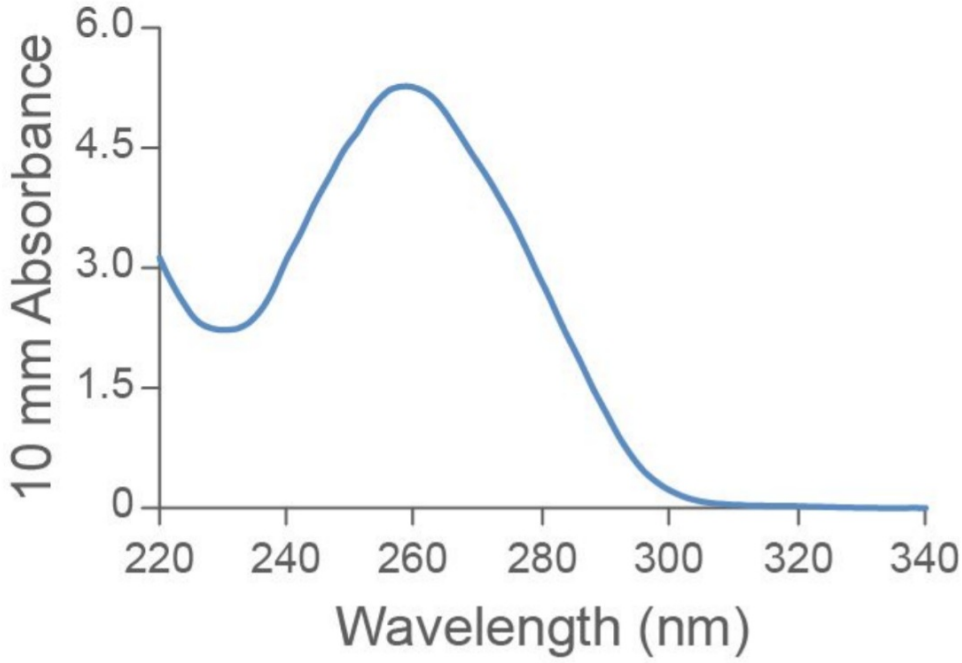
Method

1. **Caution:** This method requires the use of β-mercaptoethanol, which must be handled in a fume hood. All steps from step 5 onwards should be carried out in a fume hood until the DNA is eluted (step 13).
2. Pour the content of a sperm straw (~150–200 µl) into a 1.5 ml Eppendorf DNA LoBind tube.
3. Add 950 µl of guanidine-HCl buffer (50 mM guanidine-HCl, 10 mM Tris-HCl, 10 mM NaCl, 10 mM EDTA, 1 mM NaOH) and 50 µl of Proteinase K to the sperm sample.
4. Incubate for 10 minutes at 37°C.
5. Centrifuge the sample at 4°C at 12,000x g for 5 minutes.
6. Transfer the supernatant to a new 1.5 ml Eppendorf DNA LoBind tube and, in a fume hood, add 20 µl of Proteinase K and 20 µl of β-mercaptoethanol. Do not discard the pellet.
7. Add 300 µl of ATL buffer, 50 µl of Proteinase K, and 7 µl of β-mercaptoethanol to the pellet.
8. Incubate both fractions (steps 5 and 6) at 60°C for 2 hours and invert the tubes every 30 minutes.
9. Pool the content of both tubes in a 50 ml Falcon and add 18 ml of Buffer G2.
10. Mix and incubate at 60°C for 5 minutes to ensure the lysate is homogeneous.
11. Equilibrate one QIAGEN Genomic-tip 100/G column with 4 ml of QBT.
12. Pour the lysate through the column.

13. Purify the lysate according to the standard protocol (steps 3–6, pages 50–52).#
14. To maximize the DNA yield, we recommend to perform the elution overnight at room temperature in 150 µl of TE.
15. **Optional step:** Dilute 3 µg of DNA in 50 µl of TE and shear using a Covaris g-TUBE™ at 6000 x g, OR Dilute 3 µg of DNA in 100 µl of TE and shear using the Megaruptor® 3 (speed setting of 30).

Results

- **Yield:** 10-20 µg
- **OD 260/280:** 1.99
- **OD 260/230:** 1.60



Sequencing performance

Libraries were prepared from unsheared and sheared DNA using the Ligation Sequencing Kit.

- Typical flow cell output without shearing is lower than that with shearing. Output from the flow cell may be increased by performing a flow cell wash step (at the point where the rate of data acquisition begins to deteriorate due to the accumulation of pores in the “unavailable” or “recovering” state) and then adding a new library. Alternatively, the extracted DNA could be sheared before library preparation to increase flow cell output.
- Typical flow cell output with shearing is equivalent to the Lambda DNA supplied with the Control Expansion pack (EXP-CTL001).

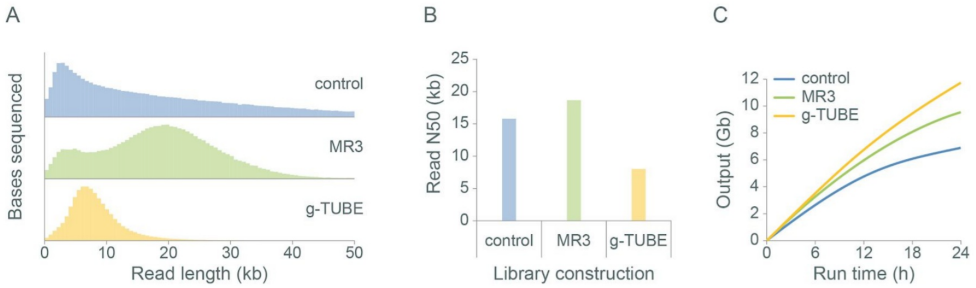


Figure 1. Performance of libraries from gDNA extracted from bull sperm when sequenced with MinION.
Genomic DNA extracted from bull sperm was sheared using either the Covaris g-TUBE™ or the Diagenode Megaruptor® 3. Sequencing libraries were prepared from unsheared DNA (control), DNA sheared using Megaruptor® 3 (MR3) and DNA sheared using a g-TUBE, and the libraries run on the MinION. Read length distributions (panel A), read N50 values (panel B) and flow cell output (panel C) are

displayed.

Change log

Version	Change
v1, 24th September 2019	Initial protocol publication
v2, 14th August 2023	Updated URL link