Promega

Product Application

Purification of High Molecular Weight DNA from Human Semen

Purify high molecular weight DNA from human semen samples with Wizard® HMW DNA Extraction Kit.

Kit: Wizard® HMW DNA Extraction Kit (Cat.# A2920)

Analyses: Absorbance, Fluorescent DNA binding dye, Pulsed-Field Gel Electrophoresis

Sample Type: Human Semen

Input: 25µl-50µl

This protocol was developed by Promega Applications Scientists and is intended for research use only.

Users are responsible for determining suitability of the protocol for their application.

For further information, see Technical Manual TM604, available at: **www.promega.com/protocols**

or contact Technical Services at: **techserv@promega.com**

Materials Required:

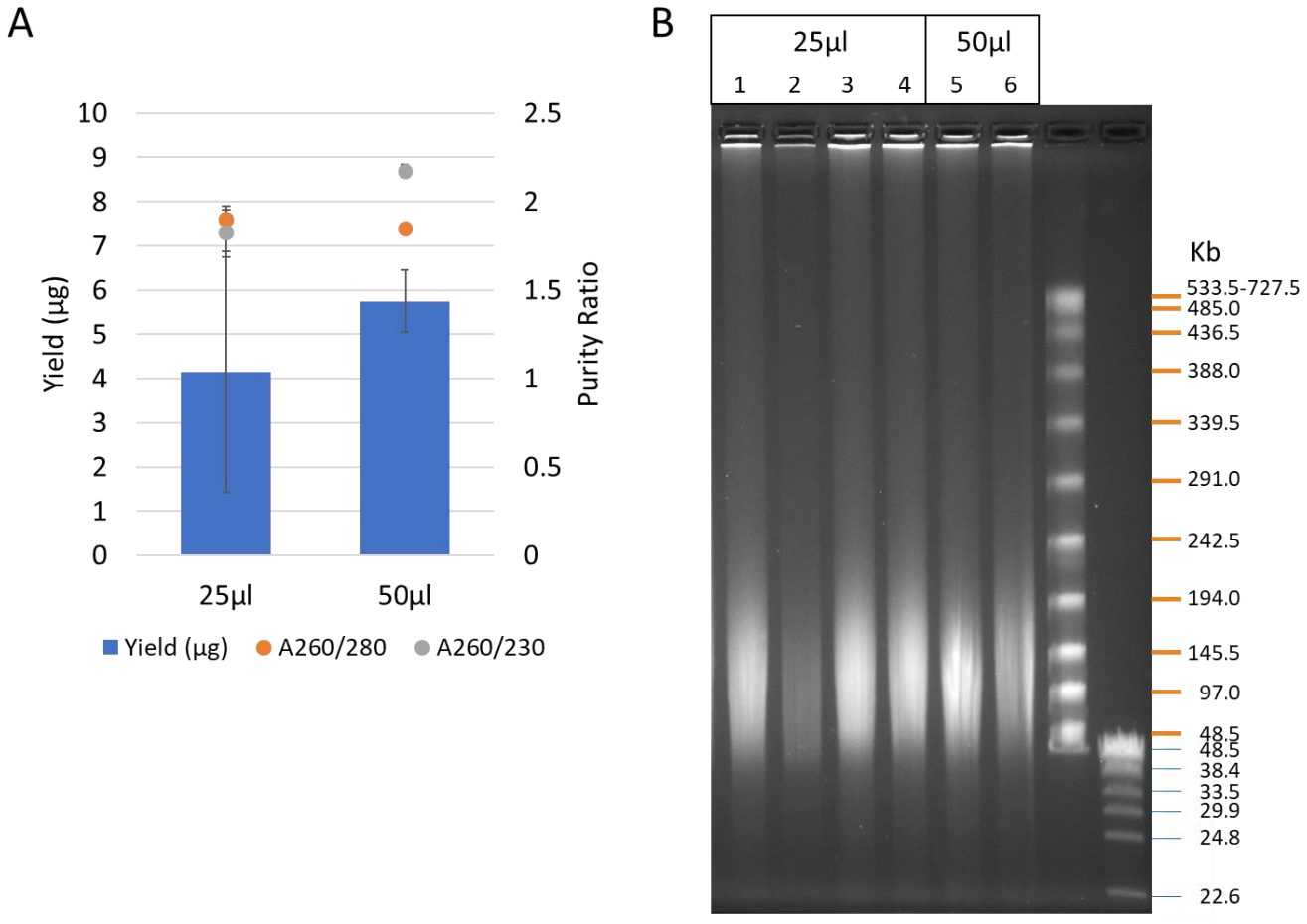
- Wizard® HMW DNA Extraction Kit (Cat.# A2920)
- 1-Thioglycerol (Cat.# A208B)
- 1.5ml microcentrifuge tubes
- Wide-bore pipette tips (1,000µl and 200µl)
- Heat blocks or water baths, set to 37°C, 56°C, and 65°C
- Isopropanol (room temperature)
- 70% ethanol (room temperature)

Protocol:

1. Transfer semen a 1.5ml tube.
2. Add 500µl of HMW Lysis Buffer A.
3. Add 10µl of 1-Thioglycerol.
4. Using 1,000µl wide bore pipette tips, mix the solution 5 times by drawing the contents slowly from the bottom of the tube, and then expelling the lysate rapidly down the side of the tube.
5. Incubate at 65°C for 30 minutes. Cool for 2 minutes.
6. Add 3µl of RNase A Solution to the lysate and mix the sample by inverting 5-7 times.
7. Incubate the mixture at 37°C for 15 minutes.
8. Add 20µl of Proteinase K Solution to each lysate and mix the sample by inverting the tube 10 times.
9. Incubate the mixture at 56°C for 15 minutes.
10. Cool to room temperature for at least 5 minutes.
11. Add 200µl of Protein Precipitation Solution to the lysate. Using 1,000µl wide bore tips, mix the solution 5 times by drawing the contents from the bottom of the tube, and then expelling the lysate rapidly down the side of the tube.
12. Incubate on ice for 5 minutes.
13. Centrifuge at 16,000 x g for 10 minutes at room temperature.
14. Slowly transfer the supernatant to a 1.5ml tube containing 600µl of isopropanol by decanting.
    - It is not necessary to transfer all of the supernatant. It is preferable to leave some behind in order to avoid aspirating the precipitated protein.
15. Gently mix the solution by inverting 8 times. Incubate for 1 minute at room temperature and repeat the inversions.
    - DNA may appear as a white mass of threads.
16. Centrifuge at 16,000 x g for 2 minutes at room temperature.

17. Carefully pipet off the supernatant, being mindful that the pellet may be loose. Some supernatant may be left behind to preserve the pellet.
18. Add 600µl of room temperature 70% Ethanol. Gently invert the tube several times to wash the pellet and tube walls.
19. Centrifuge at 16,000 x g for 2 minutes at room temperature.
20. Carefully aspirate the supernatant. Standard pipette tips may be used. Care must be taken not to aspirate the DNA pellet.
21. Invert the tube on clean absorbent paper and air-dry the pellet for 10-15 minutes.
22. Add 50µl of DNA Rehydration Solution to the tube. Incubate at room temperature overnight to rehydrate the pellet.
23. Store the DNA at 4°C.

Results:



Analyses of high molecular weight DNA from human semen. 25µl or 50µl of previously frozen semen was used for purifications with the Wizard® HMW DNA Extraction Kit, as indicated. **(A)** Yield and purity ratios of DNA purified from human semen. Concentration was measured with 1µl of DNA sample using QuantiFluor® ONE dsDNA System (Cat.# E4870) on a Quantus™ Fluorometer (Cat.# E6150). K562 Genomic DNA (Cat.# E4931) was used as a standard. Concentration was multiplied by 50µl to calculate yield in µg. Absorbance at 230, 260, and 280nm was measured on a NanoDrop™ 8000 Spectrophotometer (Thermo Fisher Scientific) and purity ratios were calculated by the NanoDrop™ software. Mean ± standard deviation is shown for all measurements, n=4 purifications for 25µl samples and n=2 purifications for 50µl samples. **(B)** Pulsed-field gel electrophoresis analysis of high molecular weight DNA from human semen. 500ng of each DNA sample was run on a 0.75% agarose/0.5X KBB Buffer gel for 16 hours using a Sage Sciences Pippin Pulse™ power source with the 5-430Kb setting. Two molecular weight size markers were run: Lambda PFG ladder (New England BioLabs, orange) and CHEF DNA Size Standard 8.3-48.5Kb, Lambda Ladder (Bio-Rad, blue).