

Purification of High Molecular Weight DNA from Bovine Blood

Purify high molecular weight DNA from bovine blood 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:	Bovine blood
Input:	300µl
Materials Required:	<ul style="list-style-type: none">▪ Wizard® HMW DNA Extraction Kit (Cat.# A2920)▪ 1.5ml microcentrifuge tubes▪ Wide-bore pipette tips (1,000µl and 200µl)▪ Phosphate-buffered saline (PBS)▪ Heat blocks or water baths, set to 37°C and 56°C▪ Isopropanol (room temperature)▪ 70% ethanol (room temperature)

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:

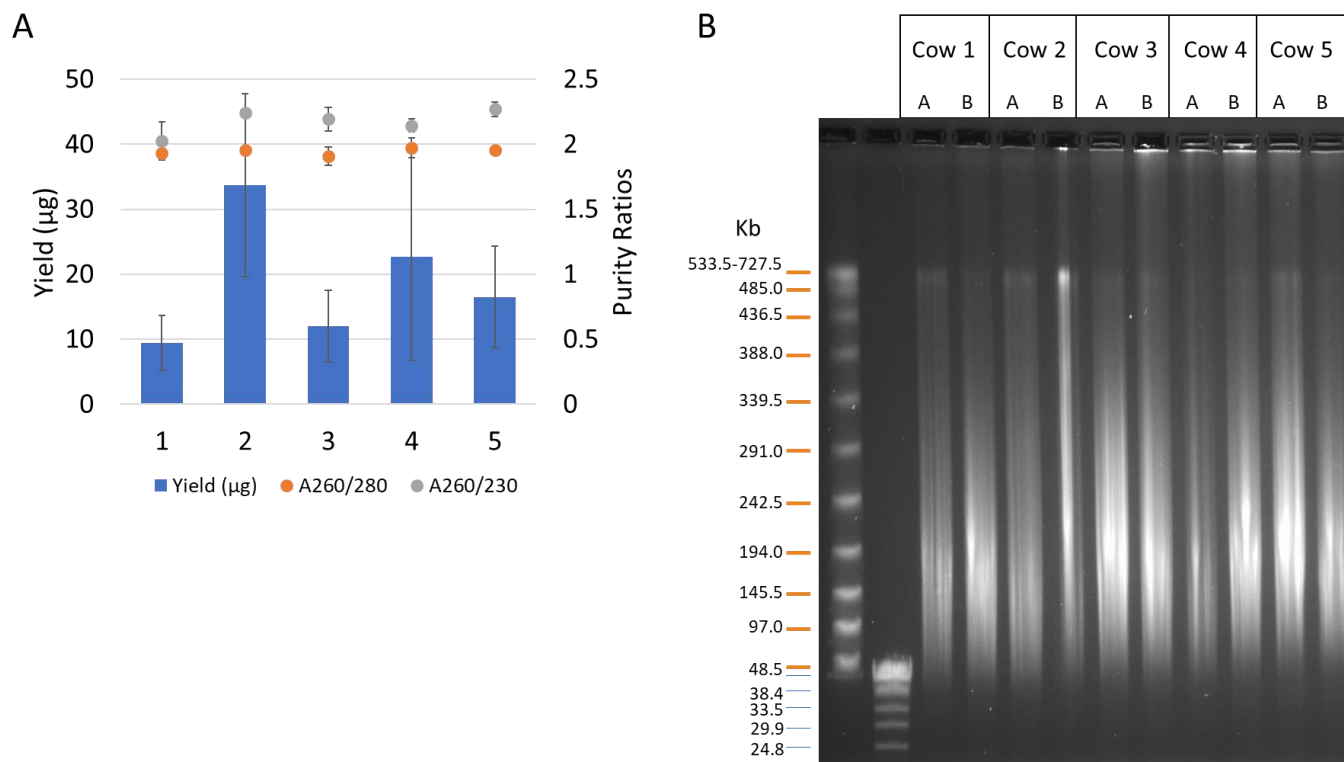
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or contact Technical Services at: techserv@promega.com

Protocol:

1. Follow HMW Genomic DNA Isolation Protocol 3.A. in Technical Manual TM604 for Isolating HMW DNA from Whole Blood to purify high molecular weight DNA from 300µl of blood.

Results:



Analyses of high molecular weight DNA from bovine blood. 300µl of blood was used for purifications with the Wizard® HMW DNA Extraction Kit. **(A)** Yield and purity ratios of DNA purified from bovine blood. 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 100µl 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=3 purifications for Cows 1-4 and n=2 purifications for Cow 5. **(B)** Pulsed-field gel electrophoresis analysis of high molecular weight DNA from bovine blood. 500ng of DNA from two purification replicates for each blood 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).