Promega

Product Application

Automated purification of DNA from horse hair samples

Purify high molecular weight genomic DNA from horse hair samples using the Maxwell® RSC Instrument and the Maxwell® RSC Blood DNA Kit.

Kit: Maxwell® RSC Blood DNA Kit (Cat.# AS1400)

Analyses: UV absorbance, dye-based quantitation, gel electrophoresis and TapeStation

Sample Type(s): Horse hair samples

Input: Fifteen horse hairs, ~ ¼ inch (6.35mm) containing the root end

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 TM419, available at: **www.promega.com/protocols**

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

Materials Required:

- Maxwell® RSC Instrument (Cat.# AS4500)
- Maxwell® RSC Blood DNA Kit (Cat.# AS1400)
- Tissue Lysis Buffer (TLA) (Cat.# A5091)
- RNase A Solution (Cat.# A7973)
- Thermomixer capable of 56°C

Protocol:

Note: To ensure the highest molecular weight DNA, avoid vortexing samples, use wide bore pipet tips, and avoid excess pipetting.

1. Cut off ~ ¼ inch (6.35mm) of fifteen hairs containing the root end and transfer to a 1.5ml tube.
2. Add 250µl of Tissue Lysis Buffer (TLA) and 35µl of Proteinase K (PK) Solution to each tube.

   Note: TLA and PK can be combined into one tube and then added to all samples.
3. Heat at 56°C with shaking at 1,000 rpm for 1 hour.
4. Add 300µl of Lysis Buffer to each sample. Mixing is not necessary.
5. Heat at 56°C with shaking at 1,000 rpm for 10 minutes.
6. Cool the samples at room temperature for 5 minutes.
7. Transfer the lysate to the well #1 of the cartridge.
8. Add 15µl of RNase A to well #1.
9. Add 50µl of Elution Buffer to the elution tube.
10. Run the Maxwell® RSC Blood DNA method on the Maxwell® RSC Instrument.

Results:

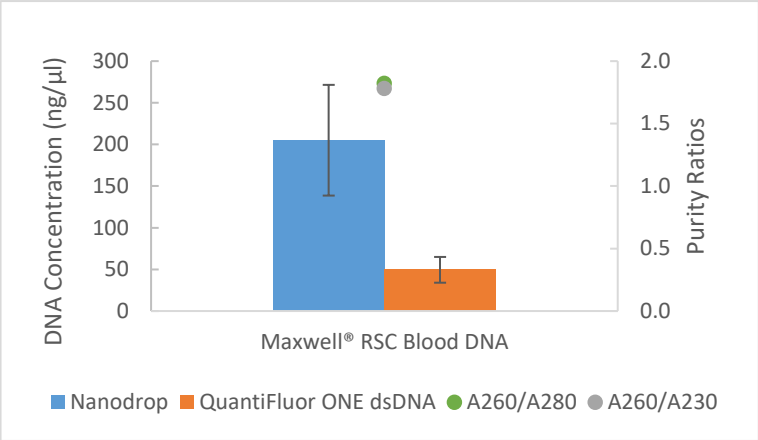
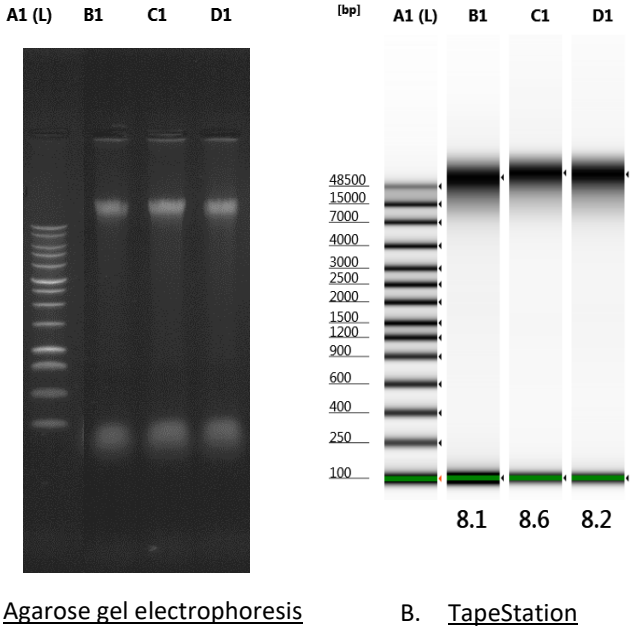


Figure 1. DNA concentration, purity ratios and yield obtained from horse hair samples using the Maxwell® RSC Blood DNA Kit (Cat.# AS1400). DNA was purified from fifteen hair samples containing the root end. DNA concentration and purity ratios were assessed by NanoDrop™ One and QuantiFluor® ONE dsDNA System (Cat.# E4871). Mean ± STD of n=3.

A1- DNA Ladder
B1- Maxwell® RSC Blood DNA replicate 1
C1- Maxwell® RSC Blood DNA replicate 2
D1- Maxwell® RSC Blood DNA replicate 3



A. Agarose gel electrophoresis B. TapeStation

Figure 2. Molecular weight of DNA extracted from horse hair samples using the Maxwell® RSC Blood DNA Kit (Cat.# AS1400) and analyzed by (A) gel electrophoresis or (B) Agilent TapeStation using the Genomic DNA ScreenTape and Reagents. DNA was purified from fifteen hair samples containing the root end. (A) 10µl of DNA eluates were analyzed on a 0.7% agarose gel in TBE with a 1kb DNA ladder, and stained with 1X SYBR Safe. (B) 1µl of DNA eluates was analyzed on an Agilent TapeStation according to the manufacturer's recommendation. The DNA integrity number (DIN) is given below each lane, and is out of a maximum value of 10.