Promega

Product Application

Automated Purification of Genomic DNA from Bird Red Blood Cells

Purify genomic DNA from bird whole blood containing nucleated red blood cells using the Maxwell® RSC Blood DNA Kit on the Maxwell® RSC Instrument.

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

Analyses:

- UV absorbance
- Dye-based quantitation
- gDNA ScreenTape Analysis
- qPCR

Sample Type(s): Red Blood Cell (RBC) pellet from bird whole blood

Input: 2.5µl to 0.625µl

Materials Required:

- Maxwell® RSC Instrument (Cat.# AS4500)
- Maxwell® RSC Blood DNA Kit (Cat.# AS1400)
- QuantiFluor® ONE dsDNA System (Cat.# E4871)
- Vortex
- Thermomixer
- 1X PBS

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**

Protocol:

1. Collect 2ml of bird whole blood with anticoagulant.
2. Centrifuge whole blood at 4,500rpm for 7 minutes at room temperature to pellet red blood cells (RBC) and remove the serum. Optional: Freeze RBC pellet at -20°C.
3. Pre-wet tip in 1X PBS by tip mixing and slowly pipet RBC pellet sample (viscous).
4. Dilute the 5µl of RBC pellet in 600µl of 1X PBS and rinse the tip by tip mixing several times.
5. Vortex to homogenize the RBC dilution.
6. Aliquot the desired RBC input in a new microtube (for example 300µl, 150µl and 75µl of the RBC dilution corresponding to 2.5µl, 1.25µl and 0.625µl of RBC pellet inputs, respectively) and adjust the volume to 300µl with 1X PBS, if necessary.
7. Add 30µl of Proteinase K and 300µl of Lysis Buffer and invert 10 times.
8. Incubate at 56°C for 20 minutes while shaking (800rpm).
9. Meanwhile, prepare cartridges as described in the Maxwell® RSC Blood DNA Kit Technical Manual (TM419).
10. Add 100µl of Elution Buffer to the elution tubes.
11. Transfer the entire lysate (630µl) to well #1 of the cartridge.
12. On the Maxwell® RSC Instrument, select the Blood DNA method, place the prepared deck tray in the instrument, and start the method.

Results:

gDNA was successfully purified from bird red blood cells with the Maxwell® RSC Blood DNA Kit. qPCR amplification of 10ng of gDNA purified from 2.5µl RBC input did not show any qPCR inhibition (data not shown).

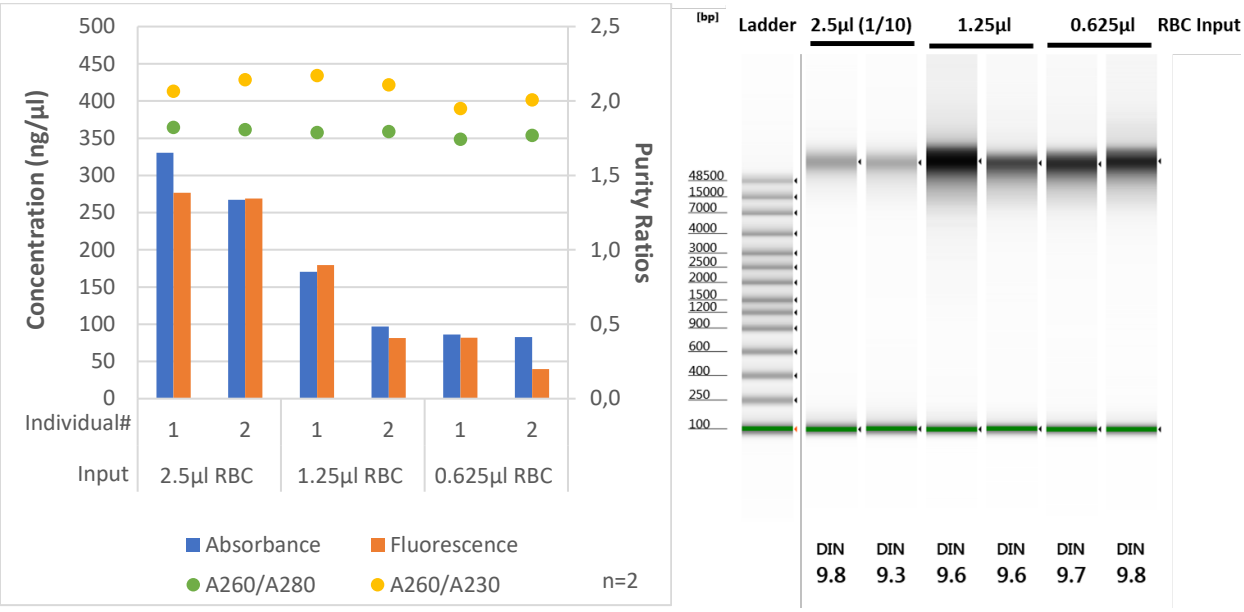

Figure 1. Concentration, purity ratios and integrity of gDNA purified from an input titration of duck RBC pellets using the Maxwell® RSC Blood DNA Kit. An input titration from 5µl to 0.625µl equivalent volumes of duck RBC pellet (collected from 2 individuals) were purified in duplicate with the Maxwell® RSC Blood DNA Kit (100µl elution volume). **Left:** DNA concentration was measured by UV absorbance on the NanoDrop™ ONE Spectrophotometer (blue) or by fluorescence with the QuantiFluor® ONE dsDNA System (Cat.# E4871) (orange). K562 genomic DNA (Cat.# E4931) was used to generate the QuantiFluor® ONE dsDNA standard curve for quantitation. Purity ratios A_{260}/A_{280} (green) and A_{260}/A_{230} (yellow) were measured by UV absorbance on the NanoDrop™ ONE Spectrophotometer. Shown are average values for n=2. **Right:** Purified gDNA was analyzed using 4200 TapeStation System with a gDNA ScreenTape according to the manufacturer's instructions (Agilent). Shown is the false gel image of purified gDNA from individual #2 with the corresponding DIN values. gDNA purified from 2.5µl RBC pellet input was diluted 10-fold to within the DIN functional concentration range.