Promega

Product Application

Automated Cell-Free DNA Purification from Large Volumes of Cell-Free Urine on the Maxwell® RSC Instrument

Purify cell-free DNA (cfDNA) from large volumes of cell-free urine using the Maxwell® RSC ccfDNA LV Plasma Kit on the Maxwell® RSC Instrument.

Kit: Maxwell® RSC ccfDNA LV Plasma Kit (Cat.# AS1840)

Analyses:

- qPCR, droplet digital PCR

Sample Type: Urine

Input: 4-22ml

Materials Required:

- Urine collection containers
- Binding Buffer (BBC) (Cat.# MC1361)
- Rotisserie Shaker
- PolyATtract System 1000 Magnetic Separation Stand (Cat.# Z5410)
- Maxwell® RSC ccfDNA LV Plasma Kit (Cat.# AS1840)
- Maxwell® RSC Instrument (Cat.# AS4500)

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 TM673, available at: **www.promega.com/protocols**

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

Protocol:

1. Collect urine in desired urine collection containers.
2. Transfer urine into a conical tube(s) and centrifuge at 2,000 x *g* for 10 minutes at 4°C.
3. Remove supernatant and transfer to a new tube, avoiding the cell pellet. This is 1x cleared urine.
4. Centrifuge 1x cleared urine at 2,000 x *g* for 10 minutes at 4°C.
5. Remove supernatant and transfer to a new tube, avoiding any cell pellet. This is 2x cleared urine.
6. Add powder EDTA to the pooled urine for a final concentration of 20mM and vortex until in solution.
    a. Cell-free urine may be stored at -80°C until use or processed fresh. If stored at -80°C, thaw and pool aliquotes prior to processing.
7. Process samples according to the Maxwell® RSC ccfDNA LV Plasma Kit Technical Manual (TM673), with the following modifications:
    a. Section 5, Step 4: Incubate for 90 minutes on a rotisserie shaker.
    b. Section 5, Step 6: Place tubes in a magnetic stand for 5 minutes to immobilize the resin. Decant the supernatant while tubes remain in the magnetic stand.
8. Prepare Maxwell® RSC Cartridges as detailed in the Technical Manual using the PCR Elution Buffer.
9. Select the Maxwell® RSC ccfDNA LV Plasma method on the Maxwell® RSC Instrument and run.

Results:

cfDNA was successfully purified from 4, 10, and 22ml of cell-free urine using the Maxwell® RSC ccfDNA LV Plasma Kit according to the protocol described above, including the step of freezing cell-free urine at -80°C. cfDNA was amplifiable in both qPCR and droplet digital PCR.

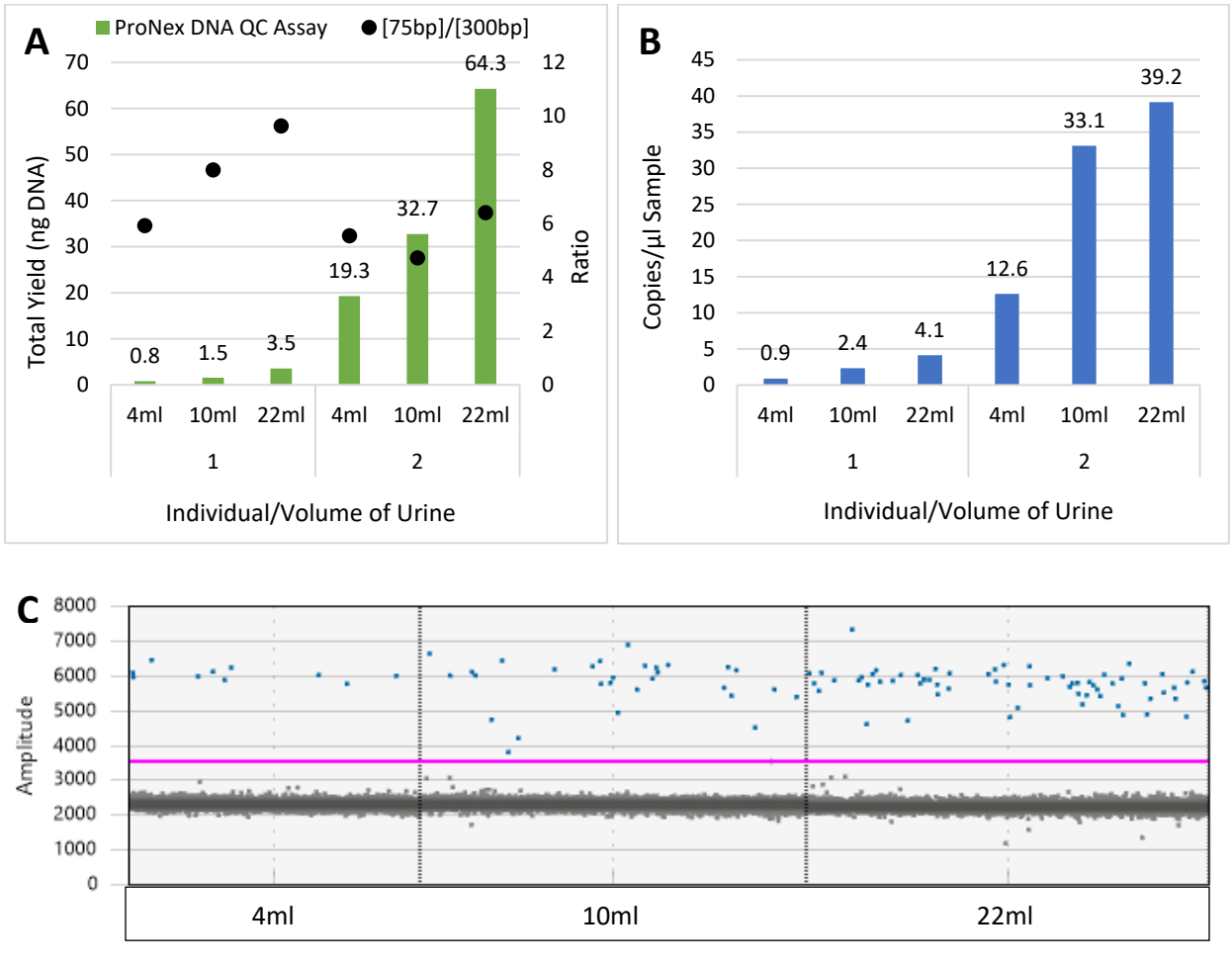

Figure 1. Yields and droplet digital PCR results of DNA purified from cell-free urine. For two individuals (1 and 2), cfDNA was purified in duplicate from 4, 10, or 22ml of cell-free urine using the Maxwell® RSC ccfDNA LV Plasma Kit (Cat.# AS1840) with the PCR Elution Buffer according to the protocol describe above. **A. Total DNA yield based on qPCR**. Purified DNA was amplified in duplicate using the ProNex® DNA QC Assay (Cat.# NG1005) on the BioRad CFX96 Touch™ Real-Time PCR Detection System. Data represent the average concentration of duplicate purifications amplified in duplicate. **B. Average EIF2C1 copies per µl of sample based on ddPCR™.** Droplet digital PCR quantitation was performed using the ddPCR™ CNV Assay EIF2C1, Hsa FAM (BioRad). Data represent average copies of EIF2C1 target amplified per 1µl of purified cfDNA eluate input for duplicate purifications. **C. Example 1D droplet plots for the EIF2C1 target for Individual 1**. The threshold, above which droplets are considered positives (contain target DNA), is represented by a pink line. The Y-axis represents relative fluorescence. Each column contains the droplet plot for the sample indicated on the X-axis. The width of the columns corresponds to the number of accepted droplets.