Promega

Product Application

Automated DNA Purification from Human Stem Cells

Purify DNA from cultured human stem cells with the Maxwell® RSC Blood DNA Kit on the Maxwell® RSC Instrument.

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

Analyses: Absorbance, Dye-based quantitation, qPCR, Oxford Nanopore Long-Read Sequencing

Sample Type: Stem cells

Materials Required:

- Maxwell® RSC Instrument (Cat.# AS4500)
- Maxwell® RSC Blood DNA Kit (Cat.# AS1400)
- 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 cultured stem cells by trypsinization.
2. Pellet cells by centrifugation at room temperature in a microfuge at 13,000–16,000 × *g* for 1–2 minutes or in a centrifuge at 2,000 × *g* for 10 minutes.
3. Remove the supernatant, being careful not to disturb the cell pellet. If no pellet is visible, avoid the position in the tube where a pellet would be expected. Low cell numbers may result in a pellet that is not visible by eye.
4. Resuspend the pellet in 100µl of PBS.
5. Add 300µl of Lysis Buffer and 30µl of Proteinase K.
6. Vortex sample to fully resuspend pellet.
7. Incubate at 56°C for 20 minutes.
8. Add entire lysate volume to well #1 of the Maxwell® cartridge.
9. Place the cartridge in the deck tray and add a plunger into well #8.
10. Add 50µl of Elution Buffer to the bottom of each Elution Tube and place on the deck tray.
11. Select the RSC Blood DNA method and proceed with the on-screen instructions to purify DNA using the Maxwell® RSC Instrument.

Results:

Amplifiable DNA was successfully purified from cultured human stem cells using the Maxwell® RSC Blood DNA Kit. Long-read sequencing was performed on the purified DNA, yielding high-quality data.

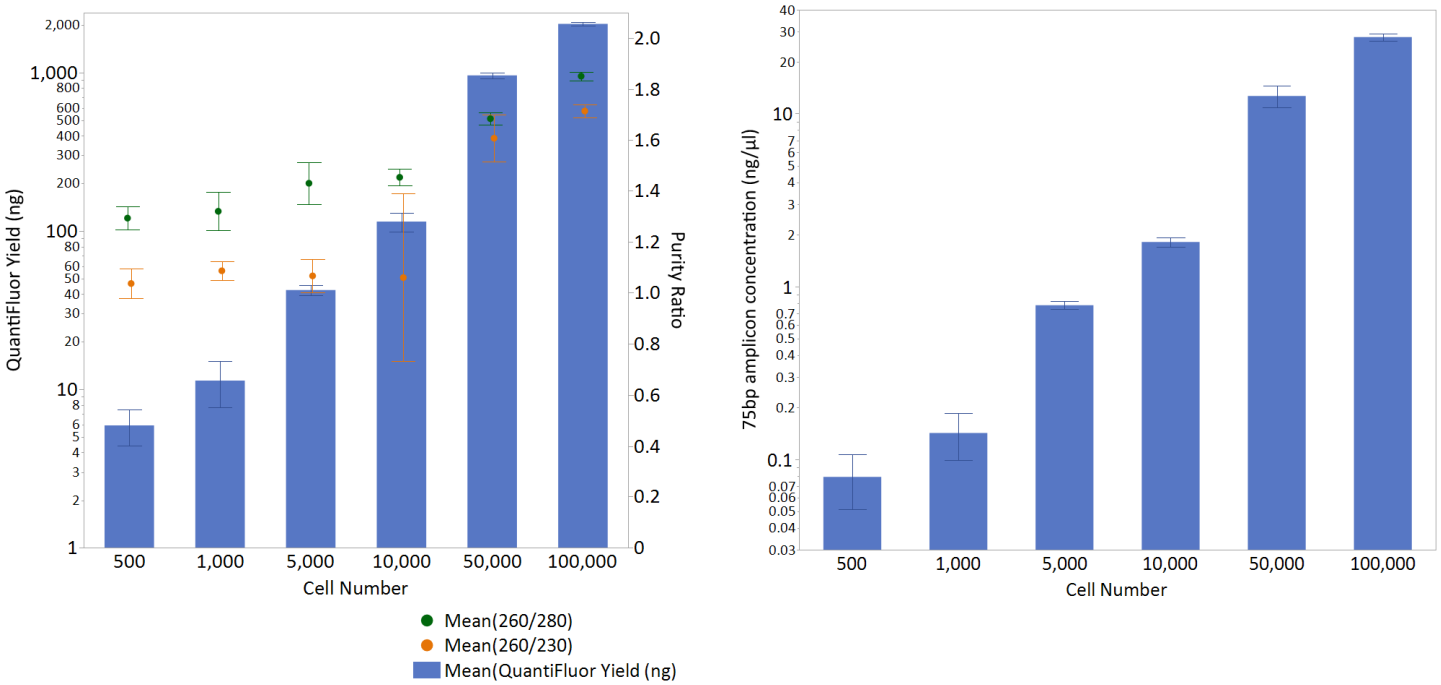

Figure 1. Yield, purity, and amplifiability of DNA purified from stem cells. WTC-11 iPS cells were cultured in mTeSR™1, trypsinized and centrifuged to produce pellets with the indicated cell numbers. Samples were purified in triplicate with the Maxwell® RSC Blood DNA Kit using the protocol described above. **Left**: Average yield and purity. Yield was calculated by multiplying concentration measured with QuantiFluor® ONE dsDNA System (Cat.# E4871) by eluate volume. Absorbance was measured with a NanoDrop™ spectrophotometer. Mean ± STD is shown for each measurement, n=3 purification replicates. **Right:** Amplifiability. 2µl of each eluate was amplified in duplicate with the ProNex® DNA QC Assay (Cat.# NG1005). Mean concentration of the 75bp target is shown ± STD, n=6 (triplicate samples amplified in duplicate).

Metric	Value
Number of Active Pores	1216
Total Yield (GB)	7.96
Reads Analyzed	1,120,761
Avg Quality Score	13.9
Mean Seq Length	7,105
Median Read Length	4,010
Maximum Read Length	125,967
N50 Read Length	14,188

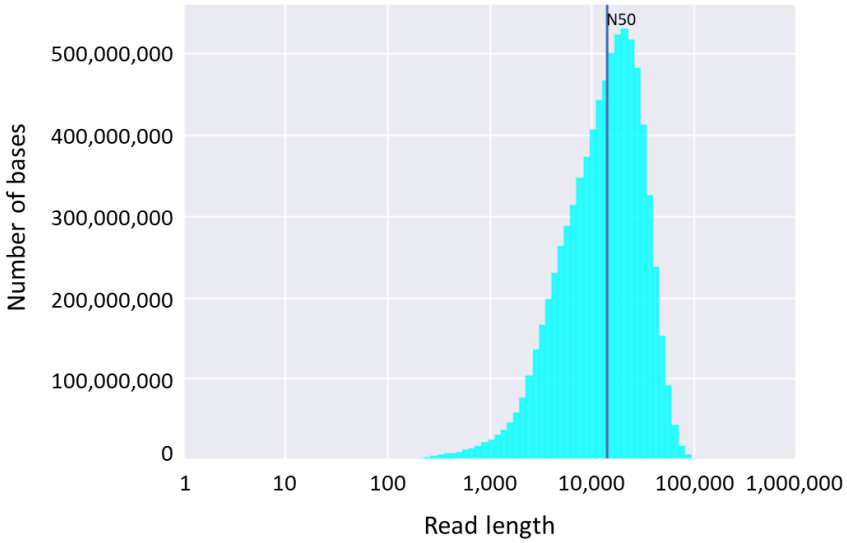

Figure 2. Long read sequencing of human stem cell DNA. 1µg of DNA was prepared for sequencing using the Oxford Nanopore Genomic DNA by Ligation (SQK-LSK110) Protocol[1] with modifications to bead clean up steps to incorporate ProNex® Size-Selective Purification System[2], and Long Fragment Buffer (LFB) was used for washes during post adapter ligation cleanup. The resulting library was run on an Oxford Nanopore MinION and base called with Guppy v6.0.1 using the high-accuracy option. Left: Sequencing quality metrics resulting from Oxford Nanopore sequencing of purified human stem cell DNA. Right: Weighted histogram of read lengths after log transformation.

References:

1. Oxford Nanopore Genomic DNA by Ligation (SQK-LSK110) Protocol. Version: GDE_9108_v110_revP_10Nov2020. Last update: 02/24/2022.
2. ProNex® Chemistry Based Clean-Up in the Oxford Nanopore Ligation Sequencing Kit. Application Note PA411.