DNA Purification from Mammalian Cell Lines Using the ReliaPrep™ Large Volume HT gDNA Isolation System

Promega Corporation

Materials Required:
- ReliaPrep™ Large Volume HT gDNA Isolation System (Cat.# A2751)
- HSM 2.0 Instrument (Cat.# A2715)
- RNase A Solution (Cat.# A7974)
- Nuclease-Free Water (Cat.# P1199)
- Tissue Lysis Buffer (TLA) (Cat.# A5091)
- 25mM Tris-HCl (pH 8.0)
- 50% ethanol

Caution: We recommend the use of gloves, lab coats and eye protection when working with these or any chemical reagents.

Protocols: *ReliaPrep™ Large Volume HT gDNA Isolation System Technical Manual,* #TM341, and *HSM 2.0 Instrument Operating Manual,* #TM389.

DNA was purified from 10–40 million cells using the ReliaPrep™ Large Volume HT gDNA Isolation System and HSM 2.0 Instrument. The resulting purified DNA was of high purity and molecular weight, and the protocol is compatible with fresh or frozen cell pellets.

Methods

Samples were processed in a semi-automated method following the protocol provided below, with the user dispensing and aspirating reagents as directed by the software on a computer screen.

1. Resuspend cell pellet (10–40 million cells) in 2ml of Nuclease-Free Water. Add cells to a 50ml conical tube. Place tube on the HSM 2.0 Instrument.
2. Select the Buccal Wash Purification Protocol on the HSM 2.0 Instrument.
3. Select Start.
4. Follow the user prompts on screen to add each reagent. Note that some required volumes differ from the volumes displayed on screen as indicated below in the table. Be sure to add the specified reagent volumes.

Reagent	Volume
Proteinase K (PK) Solution	120µl[1]
RNase A Solution	120µl[1]
Tail Lysis Buffer	3ml[1,2]
Cell Lysis Buffer (CLD)	3ml
Binding Buffer (BBA)	6ml
ReliaPrep™ Resin	500µl[1]
Prepared Wash Buffer (WBC)	5ml
Prepared Wash Buffer (WBC)	5ml
50% ethanol	4ml
25mM Tris-HCl (pH 8.0)	2ml (of which ~1.4ml will be recovered)

[1]Volumes used in this protocol differ from the volumes displayed on screen.

[2]During the buccal wash purification protocol, you will be prompted to add Tail Lysis Buffer. However, the Tail Lysis Buffer is not available separately. The compositions of the Tail Lysis Buffer and Tissue Lysis Buffer (TLA) are identical. Substitute 3ml of Tissue Lysis Buffer (TLA) for 3ml of Tail Lysis Buffer mentioned in the buccal wash purification protocol.

Results

Genomic DNA (gDNA) was purified from 10–40 million SW48, RKO or HCT116 cells using the ReliaPrep™ Large Volume HT gDNA Isolation System and HSM 2.0 Instrument as described above. DNA concentrations were estimated by measuring absorbance at 260nm using a NanoDrop® spectrophotometer, and the average concentration ranges were calculated:

Starting Material	DNA Concentration
10 million cells	29–35ng/µl
20 million cells	99–107ng/µl
30 million cells	144–146ng/µl
40 million cells	190–196ng/µl

The average DNA yield from each set of triplicate samples is shown in Figure 1.

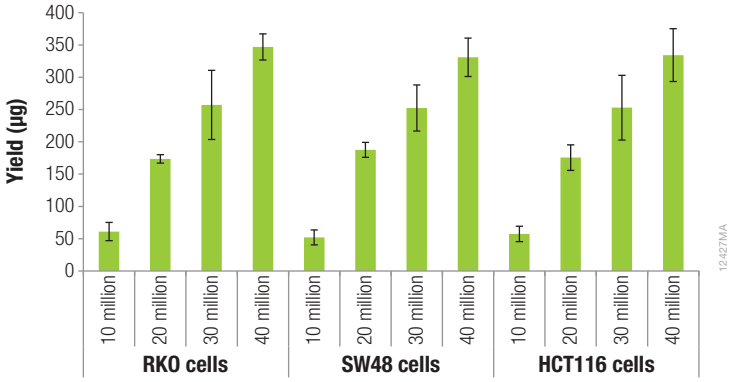

Figure 1. Yields of DNA purified from cell lines using the ReliaPrep™ Large Volume HT gDNA Isolation System. Averages of triplicate samples are shown.

To assess DNA purity, absorbance was measured at 230nm, 260nm and 280nm, and A_{260}/A_{230} and A_{260}/A_{280} ratios were calculated. The average ratios are shown in Figure 2. A_{260}/A_{280} ratios were >1.8, and most A_{260}/A_{230} ratios were in the range of 2.0–2.4, indicating the DNA was of high quality.

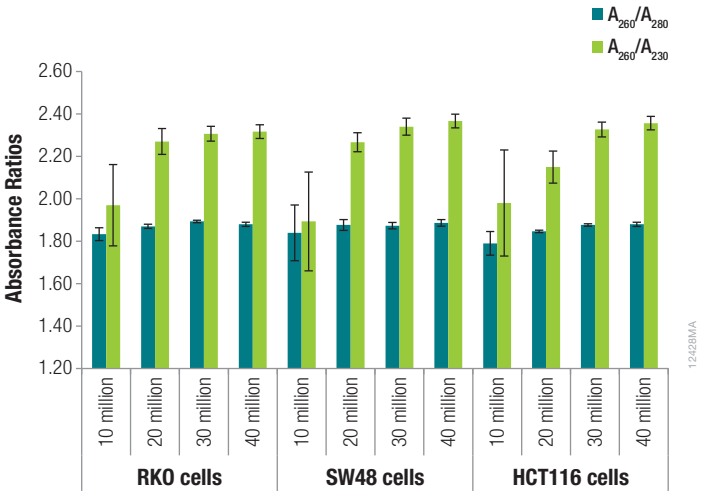

Figure 2. Purity of DNA purified from cell lines using the ReliaPrep™ Large Volume HT gDNA Isolation System. The average A_{260}/A_{230} and A_{260}/A_{280} ratios of triplicate DNA samples are shown. Absorbance was measured using a NanoDrop® spectrophotometer.

To examine DNA integrity, 200ng of DNA was analyzed by agarose gel electrophoresis (Figure 3). For all DNA samples, the fragment size was >21.2kb, indicating the DNA was intact.

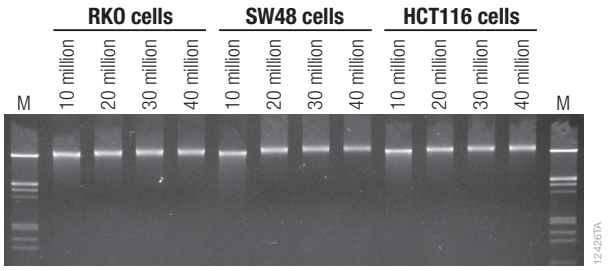

Figure 3. Gel electrophoresis of purified DNA. A volume equivalent to 200ng of each sample was loaded on a 1% agarose gel. DNA was visualized by ethidium bromide staining. M = Lambda DNA/EcoRI + HindIII Markers. The largest fragment in the marker is 21.2kb.

Conclusion

These results demonstrate the performance of the ReliaPrep™ Large Volume HT gDNA Isolation System in conjunction with the HSM 2.0 Instrument to isolate high-quality genomic DNA from 10–40 × 10^6 cells.

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