Promega

Product Application

Isolation of Genomic DNA from Mouse Tails on the Maxwell® RSC instrument

Isolate genomic DNA from mouse tail snip using Maxwell® RSC instrument and Maxwell® Tissue DNA kit using enzymatic treatment for lysis.

Kit: Maxwell® RSC Tissue DNA Kit (Cat.# AS1610)

Analyses: Absorbance, dye-based and qPCR quantitation, agarose gel electrophoresis.

Sample Type(s): Mouse tail snips

Input: 0.5cm tails snips

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 TM476, available at: **www.promega.com/protocols**

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

Materials Required:

- Maxwell® RSC Tissue DNA Kit (Cat.# AS1610)
- Tissue Lysis Buffer (Cat.# A5091)
- Proteinase K, (Cat.# MC5005)
- RNase A (Cat.# A7973)
- Thermomixer Eppendorf
- Maxwell® RSC Instrument (Cat.# AS4500)

Protocol:

1. Prepare digestion mix: 265µl Tissue Lysis Buffer (TLA Buffer), 30µl Proteinase K, 5µl RNase A (per reaction).
2. Cut mouse tail snips (0.5 cm).
3. Cut snips in half and place them into 1.5ml microtubes, add 300µl of digestion mix.
4. Heat at 55°C with vigorous agitation (Thermomixer Eppendorf, 800rpm) for 30min to 1h (until mouse tail is completely digested).
5. Centrifuge at 8000xg for 2min any remaining hair or solids to the bottom of the tube.
6. Prepare Maxwell® cartridges as described in section 4.B of the Technical Manual (TM476).
7. Add 150µl of elution buffer into elution tubes.
8. Add snip lysates into well#1 of the prepared Maxwell® cartridge and mix into the lysis buffer in well #1 by pipetting 10 times.
9. Run samples on the Maxwell® RSC using the Tissue DNA protocol.

Note: A manual homogenization protocol for tissue lysis is available.

Results:

DNA was isolated from four mouse tail snips using Maxwell® RSC Tissue DNA Kit following the above protocol. Performances of this protocol is shown below.

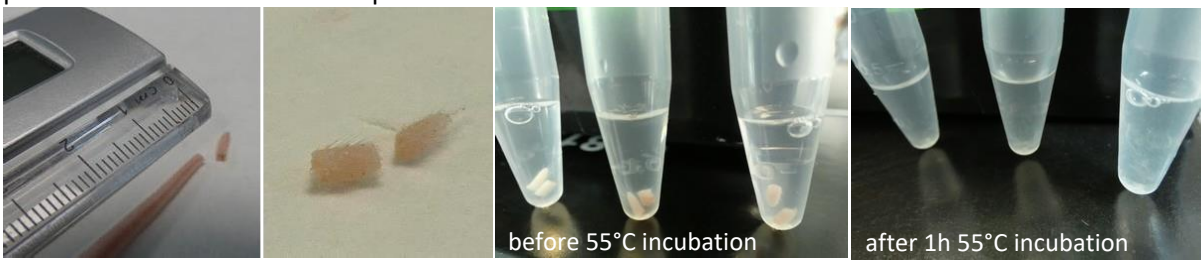

Figure 1: Mouse tail snips pre-processing. Representative images of pre-processing steps.

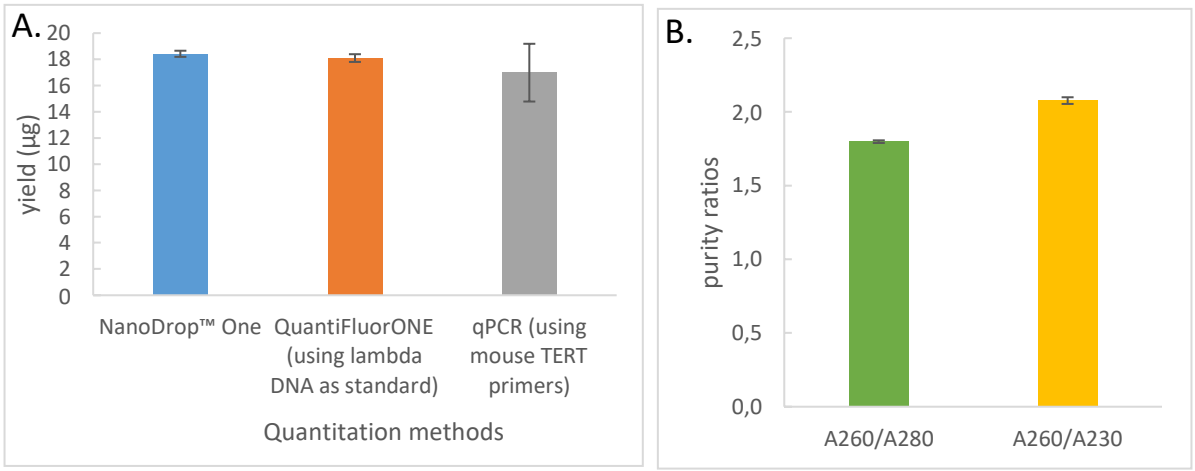

Figure 2: Yield (A) and purity (B) of DNA isolated from mouse tail snips using Maxwell® RSC Tissue DNA Kit. A. Purified DNA were quantified using absorbance (NanoDrop™ One spectrophotometer), fluorescence (QuantiFluor® ONE dsDNA System (Cat.# E4871) on a Quantus™ Fluorometer (Cat.# E6150)) and qPCR methods (Promega GoTaq® qPCR Master Mix (Cat.# A6001) on a BioRad CFX96 instrument). Average yields were calculated for 150µl elution volume. qPCR amplification was performed using mouse TERT primers with 99,1% efficiency; R^2= 0,996 and no inhibition observed on non-diluted eluates. **B.** Absorbances were read at 230, 260, and 280nm on a NanoDrop™ One Spectrophotometer and ratios were calculated to access purity. Shown is the average ± standard deviation for 4 replicates.

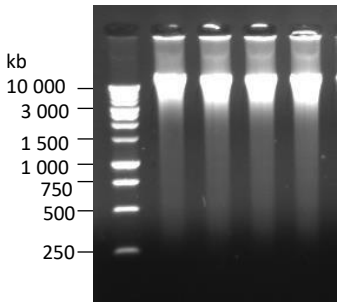

Figure 3: Agarose gel migration of DNA isolated from mouse tail snips using Maxwell® RSC Tissue DNA Kit. 5 µl of elution were loaded on a 1% agarose gel using 1kb ladder.