Promega

Product Application

Automated Purification of Mouse Tail DNA from XpressAmp™ Lysates

Purify DNA from mouse tail lysates prepared with XpressAmp™ Direct Amplification Reagents using the Maxwell® RSC Blood DNA Kit on the Maxwell® RSC Instrument. This workflow will allow for long-term storage of DNA from limited sample types and more sensitive downstream applications.

Kit:

- XpressAmp™ Direct Amplification Reagents (Cat.# A8882)
- Maxwell® RSC Blood DNA Kit (Cat.# AS1400)

Analyses:

- Dye-based quantification
- Sanger Sequencing

Sample Type(s): Mouse tail snip

Input: 1-10mg of tissue (1 tail snip of 2-4mm)

Materials Required:

- Materials for creating an XpressAmp™ lysate from mouse tissues as indicated in PA680
- XpressAmp™ Direct Amplification Reagents (Cat.# A8882)
- Maxwell® RSC Blood DNA Kit (Cat.# AS1400)
- 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 Manuals TM647 and TM419, available at: **www.promega.com/protocols**

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

Protocol:

See AppNote PA680 for creating an XpressAmp™ lysate for direct amplification from mouse tissues. The following protocol allows for further purification of DNA from XpressAmp™ lysates.

1. Add 300µl of Lysis Buffer from the Maxwell® RSC Blood DNA Kit to the remaining volume of mouse tail XpressAmp™ lysate (approximately 50µl).
2. Vortex each tube for 10 seconds.
3. Incubate at 56°C for 20 minutes.
4. During incubation, prepare the RSC cartridges as described in the Maxwell® RSC Blood DNA Kit Technical Manual (TM419):
    a. Place a plunger in well #8 of each cartridge.
    b. Add 50µl of Elution Buffer to each Elution Tube.
5. Transfer the entire lysate into well #1 of each cartridge.
6. Select the Maxwell® RSC Blood DNA method and run.

Results:

DNA was directly amplified from mouse tails by preparing lysates with XpressAmp™ Direct Amplification Reagents. DNA was then successfully purified from the mouse tail XpressAmp™ lysates using the Maxwell® RSC Blood DNA Kit. Purified DNA was suitable for Sanger sequencing using the ProDye™ Terminator Sequencing System.

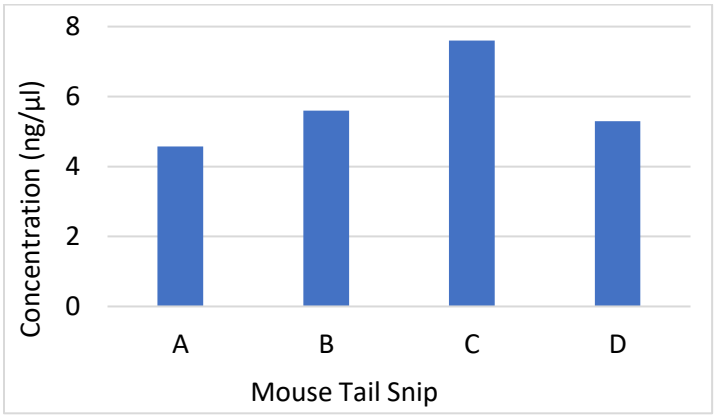

Figure 1. Concentration of DNA purified from mouse tail XpressAmp™ lysates. Lysates were prepared from mouse tail snips (2-4mm, 1.1-3.3mg) using XpressAmp™ Direct Amplification Reagents (Cat.# A8882) as described above. DNA was purified from each mouse tail XpressAmp™ lysate using the Maxwell® RSC Blood DNA Kit (Cat.# AS1400). The eluate DNA concentrations were measured using the QuantiFluor® ONE dsDNA System (Cat.# E4871) on the Quantus™ Fluorometer (Cat.# M7422). Data represent the concentration of each eluate.

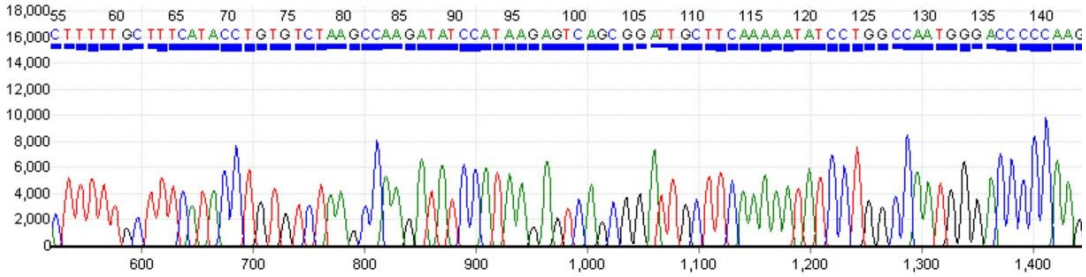

Figure 2. Example electropherogram of mTERT amplicon sequencing from DNA purified from mouse tail XpressAmp™ lysates. Lysates were prepared from mouse tail snips (2-4mm, 1.1-3.3mg) using XpressAmp™ Direct Amplification Reagents (Cat.# A8882) as described above. DNA was purified from each mouse tail XpressAmp™ lysate using the Maxwell® RSC Blood DNA Kit (Cat.# AS1400). A 319bp amplicon of mouse TERT was amplified from purified DNA using M-13 tailed primers, cleaned up with the ReliaPrep™ DNA Clean-Up and Concentration System (Cat.# A2891), and sequenced using the ProDye™ Terminator Sequencing System (Cat.# CR4302). The sequencing reactions were purified by ethanol precipitation and analyzed by capillary electrophoresis on the Spectrum Compact CE System (Cat.# CE1304). Data were viewed using the ProView™ software.

References:

1. Applications Note: Direct Amplification of DNA from Rodent Tail Snips and Ear Punches. PA680.