

DNA Purification from Cattle Ear Punches and Hair using Maxwell® RSC Tissue DNA Kit

Purify high quality DNA from cattle hair and ear punch samples collected in TSU tubes using Maxwell® RSC Tissue DNA Kit (Cat.# AS1610).

Kit:	Maxwell® RSC Tissue DNA Kit (Cat.# AS1610)
Analyses:	UV absorbance, dye-based quantitation, qPCR,
Sample Type(s):	Cattle hair and ear punches in Tissue Sampling Unit (TSU) tubes.
Materials Required:	<ul style="list-style-type: none">▪ Maxwell® RSC Tissue DNA Kit (Cat.# AS1610)▪ Tissue Lysis Buffer (TLA) (Cat.# AS5091)▪ Proteinase K Solution (Cat.# MC5005)▪ Thermomixer

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

Protocol:

1. For cattle ear punches in TSU tubes, transfer the tissue sample using forceps to a 1.5mL microtube. For cattle hair, cut off ~ ¼ inch of hair sample containing the root end and transfer to a 1.5mL microtube.
2. Add 300µL Tissue Lysis Buffer and 30µL Proteinase K (PK) Solution to each sample.
3. Incubate samples at 56°C with mixing (1000 rpm) for 1 hour.
4. Remove the samples from the incubator and transfer entire volume into well #1 of the Maxwell® RSC Tissue DNA Kit cartridge.
5. Run the Maxwell® RSC Instrument according to the technical manual.

Results:

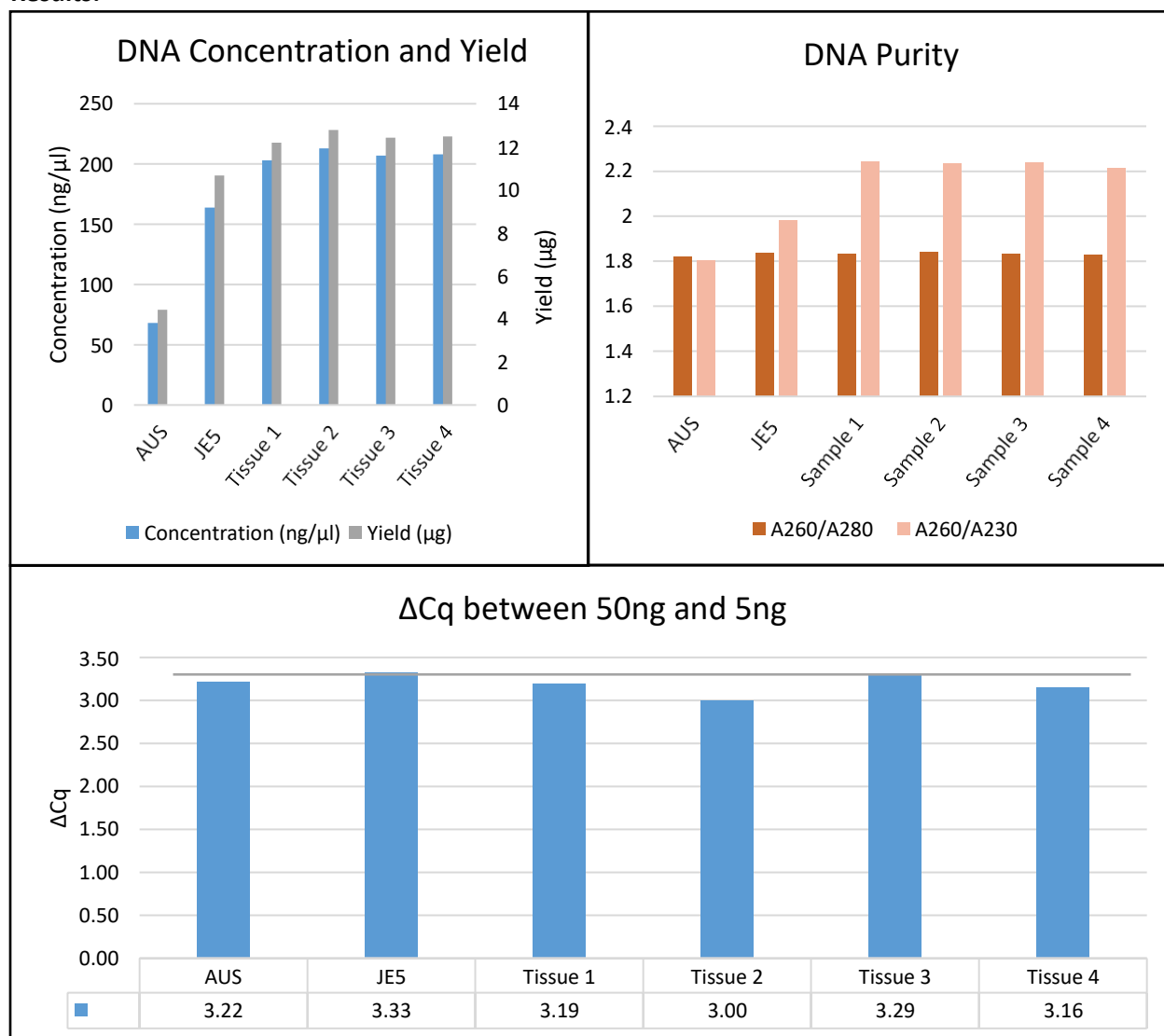


Figure 1: Top Left: DNA concentration and yield measured with QuantiFluor® ONE dsDNA System (Cat.# E4871). 2μL of eluate from each sample was quantified using the Quantus™ Fluorometer (Cat.# E6150) and QuantiFluor® ONE dsDNA System (Cat.# E4871). The tissue samples had higher yield and recovery compared to the hair, which displayed a wide deviation between hair samples (AU5, JE5) and ear punches (Tissue 1-4).

Top Right: A260/A280 and A260/A230 ratios measured by UV absorbance. 2μL of eluate was assayed to measure contamination. A260/A280 ratios are all acceptable (1.8 ideal for DNA). A260/A230 ratios are all between 1.8 and 2.2 an acceptable range.

Bottom: ΔCq between 50ng and 5ng. DNA eluates were diluted to 25ng/μL and 2.5ng/μL. 2μL of each were used in a qPCR with 400nM Universal Meat Primers and GoTaq® qPCR Master Mix (Cat.# A6001) and run under standard conditions listed in TM318; the ΔCqs are displayed above. All Cq shifts were acceptable (ΔCq of 3.3 is ideal), indicating lack of inhibition.