

promega Application Notes

Purification of Genomic DNA from *Danio rerio* Using the Maxwell® 16 System

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Abstract

The Maxwell® 16 System combines compact instrumentation, optimized automated methods, pre-filled reagent cartridges, service and support. The system provides time savings, enhanced productivity, and improved consistency of results. We have tested the utility of the system to extract genomic DNA from *Danio rerio*.

Introduction

Genomic DNA is frequently isolated from model organisms, such as the zebrafish *Danio rerio*, for use in a variety of downstream applications. Applications include cloning, nucleic acid amplification and mutation analysis. This study examines the utility of the Maxwell® 16 System for the extraction of genomic DNA from zebrafish for use in real-time PCR.

Genomic DNA Isolation

D. rerio were obtained from Carolina Biological Supply Company. One fish weighed about 220mg. Fifty milligram samples were cut from the whole fish for processing and placed directly into the Maxwell® 16 Tissue DNA Purification Kit (Cat.# AS1030) sample cartridge. Genomic DNA was purified using the Maxwell® 16 Instrument (Cat.# AS1000; firmware version 1) as described in the Technical Manual #TM284. DNA yield was determined using PicoGreen® reagent.

Quantitative PCR Analysis

Real-time PCR was performed on aliquots from two samples of purified genomic DNA using the Plexor™ qPCR System (Cat.# A4011) and primers targeting the *D. rerio* p53 gene (GenBank® Accession #AL_928875). Data were generated on an Applied Biosystems 7500 Real-Time PCR System and analyzed with the Plexor™ Analysis Software. Primers were obtained from Biosearch Technologies.

Results and Conclusions

Starting with 50mg of tissue, we obtained an average of 24.8µg of DNA (n = 10).

As observed in Figure 1, the C_t for p53 gene was approximately 26.7 when amplifying 1µl aliquots of the purified DNA (red) and 24.5 when using 5µl aliquots (green). No product accumulated in the no-template controls (black).

D. rerio genomic DNA purified with the Maxwell® 16 System is of sufficient quality and quantity to be used in real-time PCR assays.

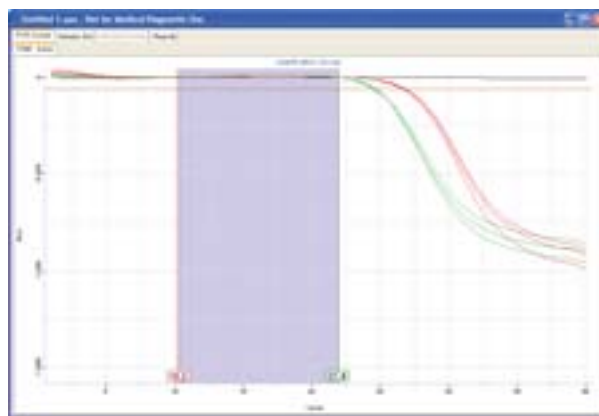


Figure 1. Real-time amplification using the Plexor™ qPCR System.

Typical Yields of Genomic DNA Using the Maxwell® 16 Tissue DNA Purification Kit.

Sample	Source	Starting Material	DNA Yield (µg)	Time Required for Processing Steps
<i>Danio rerio</i>	Dissected tissue	50mg	24.80	—
<i>Caenorhabditis elegans</i>	Whole worms	~50,000 worms	0.08	—
<i>Drosophila melanogaster</i>	Anesthetized fly	5 flies	1.52	—
<i>Drosophila melanogaster</i>	Anesthetized fly	1 fly	0.32	—
<i>Artemia franciscana</i>	Whole shrimp	1 shrimp (3–4mm)	1.72	—
<i>Saccharomyces cerevisiae</i>	Yeast colony	1 colony (3mm)	0.72	3 hours
<i>Candida albicans</i>	Yeast colony	1 colony (3mm)	3.2	—

Protocols

- ◆ Maxwell® 16 Instrument Operating Manual #TM274, Promega Corporation.
www.promega.com/tbs/tm274/tm274.html
- ◆ Maxwell® 16 DNA Purification Kits Technical Manual #TM284, Promega Corporation.
www.promega.com/tbs/tm284/tm284.html

System Information

www.promega.com/maxwell16/

Ordering Information

Product	Size	Cat. #
Maxwell® 16 Instrument*	1 each	AS1000
Maxwell® 16 Tissue DNA Purification Kit	48 preps	AS1030

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