Promega

Product Application

Automated Purification of DNA and RNA from a Single FFPE Sample

Purify DNA and RNA from a single FFPE lysate using the Maxwell® RSC FFPE DNA and RNA Kit on the Maxwell® RSC Instrument.

Kit: Maxwell® RSC DNA FFPE Kit and Maxwell® RSC RNA FFPE Kit (Cat.# AS1450 and AS1440)

Analyses: qPCR and RT-qPCR

Sample Type(s): FFPE tissue sections

Input: 10µm sections

Materials Required:

- Maxwell® RSC DNA FFPE Kit (Cat.# AS1450)
- Maxwell® RSC RNA FFPE Kit (Cat.# AS1440)
- Maxwell® RSC Instrument (Cat.# AS4500)
- Heat Block (set at 56°C and 80°C)

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 TM437 and TM436, available at: **www.promega.com/protocols**

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

Protocol:

1. Add 300µl of mineral oil to FFPE section in a 1.5ml microcentrifuge tube. Vortex for 10 seconds.
2. Heat the samples at 80°C for 2 minutes.
3. Prepare a lysis master mix of 224µl Lysis Buffer, 25µl Proteinase K and 1µl Blue Dye per sample.
4. Add 250µl of the lysis master mix to each sample tube, and vortex for 5 seconds.
5. Centrifuge samples at 10,000 *x g* for 30 seconds. Mix sample by pipetting if there is a pellet present.
6. Transfer the sample tubes to 56°C heat block and incubate for 30 minutes.
7. Transfer the sample tubes to 80°C heat block and incubate for 2 hours.
8. Remove samples and cool to room temperature for 15 minutes.
9. Centrifuge samples at max speed for 2 minutes.
10. Split blue, aqueous phase into two microfuge tubes (~120µl each)
    a. RNase Treatment (Tube #1 – DNA sample)
        i. Add 5µl RNase A to the aqueous phase lysate (blue layer), mix by pipetting.
        ii. Incubate at room temperature for 5 minutes.
    b. DNase Treatment (Tube #2 – RNA sample)
        i. Create DNase cocktail with 13µl $MnCl_2$, 7µl DNase Buffer, and 5µl DNase I per lysate.
        ii. Add 25µl of the cocktail to the aqueous phase lysate (blue layer) and mix by pipetting.
        iii. Incubate at room temperature for 15 minutes.
11. Set up two Maxwell® RSC FFPE cartridges (one from the FFPE DNA kit and the other from the FFPE RNA kit). Transfer the lysate from Tube #1 (contains DNA) into well #1 of a Maxwell® DNA FFPE Cartridge and transfer lysate from Tube #2 (contains RNA) into well #1 of a Maxwell® RNA FFPE cartridge.
12. Elute in 50µl of Nuclease-Free Water.
13. Start the method: either the Maxwell® RSC DNA FFPE or RNA FFPE method on the Maxwell® RSC Instrument, depending on nucleic acid species.

Results

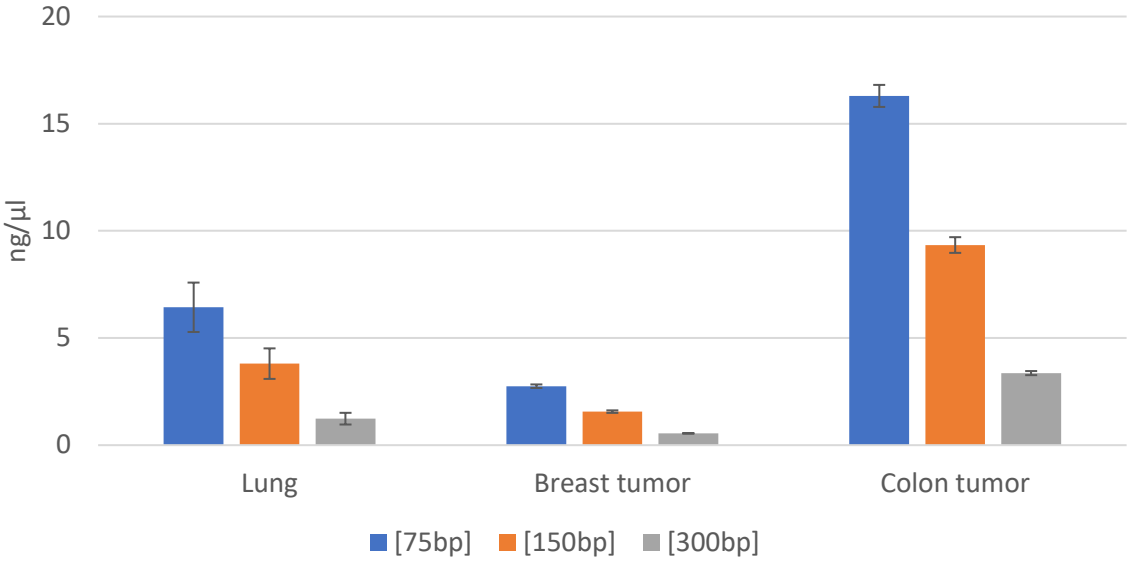

Figure 1. Concentration of DNA isolated from FFPE sections (three tissue types). DNA concentrations were quantified using ProNex® DNA QC Assay (Cat.# NG1002) on an ABI 7500 Real Time System (Applied Biosystems). The concentrations of the three targets (75bp, 150bp and 300bp) were determined relative to a human gDNA standard curve. Data are shown as mean ± STD of n=3.

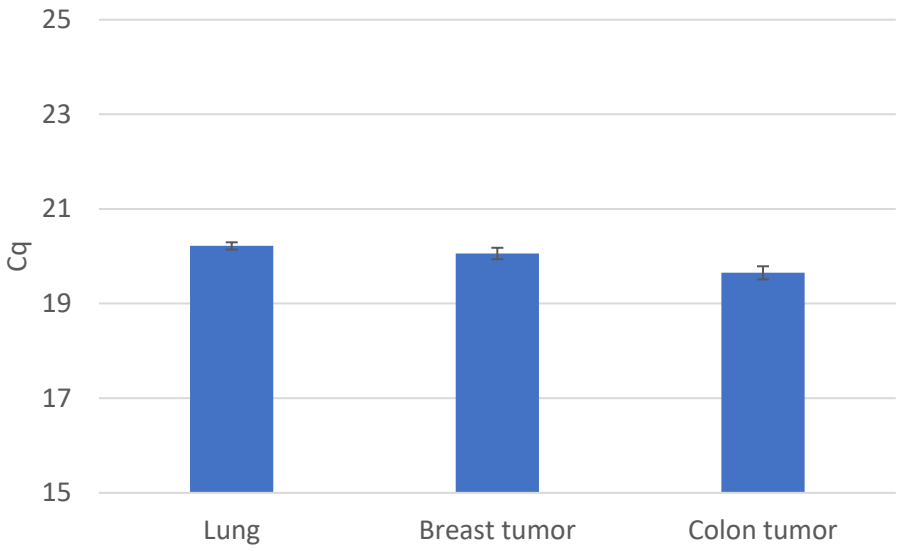

Figure 2. Cq values from RT-qPCR with RNA isolated from FFPE sections (three tissue types). RNA was amplified with primers targeting β-2-Microglobulin (B2M) mRNA using the GoTaq® 1-Step RT-qPCR System (Cat.# A6020) on an ABI 7500 Real Time System (Applied Biosystems). Data are shown as mean ± STD of n=3.