

Automated RNA Purification from FFPE Tissues using Maxwell[®] 16

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Promega
November 2010

1. The Maxwell[®] 16 Instrument

The Maxwell[®] 16 instrument extracts nucleic acid using novel paramagnetic particles, allowing optimal capture, washing and elution of the target material. Because there are no tubes or pipette tips involved, there are no clogs, drips, splashing or aerosols, greatly reducing any contamination risk. The instrument processes up to 16 samples in approximately 40 minutes, depending on which sample type is being processed.

The Maxwell[®] 16 instrument is preprogrammed with numerous purification protocols, which, combined with pre-dispensed reagent cartridges, maximizes ease and convenience. Simply add samples or lysate directly to the prefilled reagent cartridges, and press start. The optimized reagents are designed for specified sample types and deliver maximum yield and purity.

2. Prototype FFPE RNA Purification Method

The following is a protocol for the purification of RNA from formalin-fixed, paraffin-embedded (FFPE) tissue sections. The method uses a modified Low Elution Volume (LEV) cartridge and the Maxwell[®] 16 Instrument configured with the LEV hardware. The purified nucleic acid can be used directly in a variety of downstream applications, such as real-time PCR.

3. Materials Needed (48 samples)

Custom cartridge components:

- Maxwell[®] 16 LEV Cartridges
- LEV Plungers
- 0.5ml Elution Tubes
- Mineral Oil
- Lysis Solution
- Proteinase K
- Maxwell[®] RNA Resin
- Binding Buffer
- Wash Solution
- Nuclease-Free Water

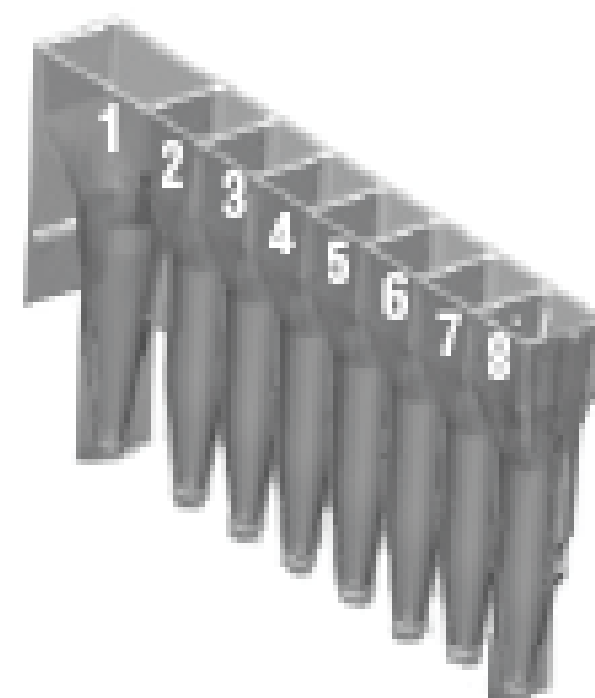
Not supplied:

- Maxwell[®] 16 Instrument
- 80°C incubator
- 56°C incubator
- 1.5ml microtubes
- 100% ethanol
- 95% ethanol



4. Preparation of LEV Cartridge

The modified LEV cartridge:



- Well 1 – 320µl of Binding Buffer, 720µl of 100% EtOH
- Well 2 – 250µl of Maxwell[®] RNA Resin
- Well 3 – 120µl of Binding Buffer, 270µl of 100% EtOH
- Well 4 – 400µl of Wash Solution
- Well 5 – 400µl of Wash Solution
- Well 6 – 400µl of Wash Solution
- Well 7 – 500µl of Nuclease-Free Water
- Well 8 – LEV Plunger

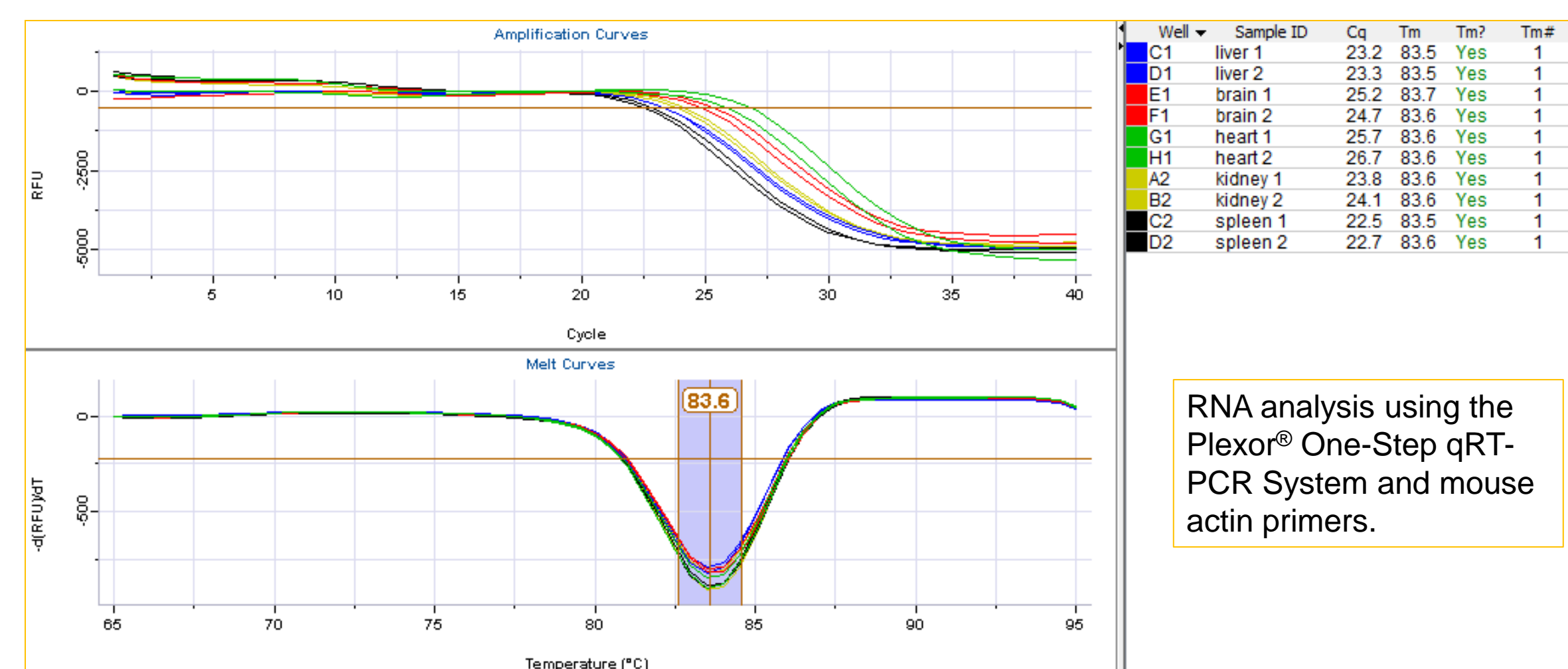
5. Protocol

1. Add 300µl of Mineral Oil to FFPE section(s) in a 1.5ml tube . Vortex.
2. Incubate at 80°C for 1 minute.
3. Add 140µl of Nuclei Lysis Solution and 20µl Proteinase K. Vortex.
4. Incubate at 56°C for 15 minutes.
5. Transfer to 80°C and incubate for 15 minutes.
6. Add entire contents to well 1 of prepared LEV cartridge.

- Process using the Maxwell[®] 16 in Research Mode using the LEV Blood method and elute in 50µl Nuclease-Free Water
- Once the extraction is complete, remove and cap each elution tube. Store RNA at -80°C until use.

6. Purification from Various FFPE Tissues

RNA was purified from FFPE mouse liver, brain, heart, kidney, and spleen tissues using the Maxwell[®] 16 Instrument. A single 10µm section of tissue was used for each purification.

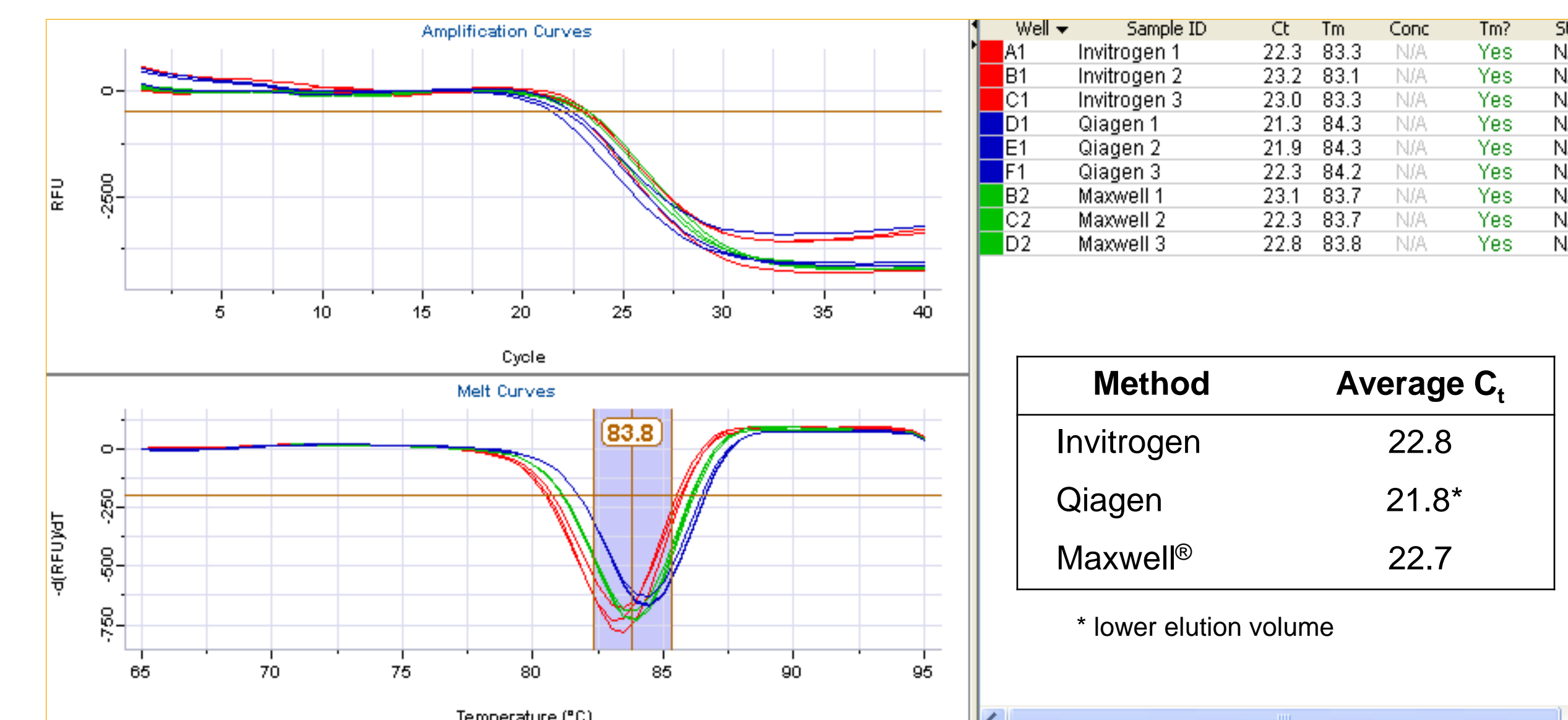


RNA analysis using the Plexor[®] One-Step qRT-PCR System and mouse actin primers.

7. Automated Method Equivalent to Manual Preps

RNA purification from FFPE mouse liver tissue using the Maxwell[®] 16 Instrument was compared with two manual purification kits – Qiagen RNeasy FFPE Kit and PureLink[™] FFPE Total RNA isolation Kit (Invitrogen). A single 10µm section of liver tissue was used for each purification.

Maxwell[®] and Invitrogen samples were eluted in 50µl. Qiagen samples were eluted in 30µl. Since Qiagen samples were eluted in a lower elution volume, C_t averages with Qiagen were expected to be ~1 C_t lower if an equivalent yield of RNA was recovered. This was reflected in the average C_t calculated for each method.



8. Summary

- Simple 6 step protocol for RNA purification from FFPE samples
- No xylene or phenol extraction required
- Purify RNA from up to 16 samples at one time using the Maxwell[®] 16 Instrument – process more samples in less time (less than 90 minutes)
- Automated purification decreases hands on time spent manually extracting and reduces the risk of RNase contamination
- Can be used with a variety of tissue sample types
- Results equivalent to manual purification methods commonly used

Protocols for a variety of new samples are being developed. Visit: www.promega.com/maxwell16/ for up-to-date information.