Isolation of high quality miRNA from decalcified, formalin-fixed, paraffin-embedded bone marrow biopsy samples

Jonathan A. Schumacher, Mohamed E Salama, Philippe Szankasi, Albert K Ho, Todd W Kelley

1 ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT 2 Department of Pathology, University of Utah, Salt Lake City, UT

ABSTRACT

Background: Decalcified, formalin fixed, paraffin embedded (D-FFPE) bone marrow biopsy samples present a challenge for molecular testing due to nucleic acid degradation. However, the integrity of micro RNA (miRNA) in D-FFPE tissues has not been well characterized. We evaluated the ability to obtain high quality miRNA from D-FFPE bone marrow biopsies that could be quantified by an array-based real-time PCR technique.

Design: Thirty D-FFPE bone marrow biopsy samples from the hemopathology archives (2003-present) were de-identified. Total RNA was extracted using either Qiagen miRNeasy FFPE or Ambion RecoverAll™ kits. Absorbance measurements at 260, 260, and 230 nm were performed to assess RNA concentration and purity. Following the manufacturer’s protocol, total RNA (50-100ng) was reverse transcribed and subjected to quantitative RT-PCR to measure 92 miRNAs using the miFinder RT™ miRNA PCR array from SA Biosciences. Relative expression values were calculated using the ΔΔCt calculation with data normalized to the average of the geometric mean of 4 housekeeping miRNAs.

Results: Bone marrow biopsy samples fixed in EDTA and decalcified with ImmunoFlon followed by tissue processing and paraffin-embedding

- Decalcified, formalin fixed, paraffin embedded (D-FFPE) bone marrow biopsy samples present a challenge for molecular testing due to nucleic acid degradation. However, the integrity of micro RNA (miRNA) in D-FFPE bone marrow biopsy samples has not been well characterized.
- We evaluated the ability to obtain high quality miRNA from D-FFPE bone marrow biopsies that could be quantified by an array-based real-time PCR technique.

- Thirty D-FFPE bone marrow biopsy samples from the hemopathology archives (2003-present) were de-identified. Total RNA was extracted using either Qiagen miRNeasy FFPE or Ambion RecoverAll™ kits.
- Absorbance measurements at 260, 260, and 230 nm were performed to assess RNA concentration and purity. Following the manufacturer’s protocol, total RNA (50-100ng) was reverse transcribed and subjected to quantitative RT-PCR to measure 92 miRNAs using the miFinder RT™ miRNA PCR array from SA Biosciences. Relative expression values were calculated using the ΔΔCt calculation with data normalized to the average of the geometric mean of 4 housekeeping miRNAs.

- Four cases were excluded from analysis due to low RNA yields (<9ng/µL). The remaining 29 cases yielded a mean RNA concentration of 79.4ng/µL (range 60.1-184ng/µL) and mean 260/280 ratio of 1.64 (range 1.09-2.15). Each case was reverse transcribed and subjected to quantitative RT-PCR to measure 92 miRNAs using the miFinder RT™ miRNA PCR array from SA Biosciences. Relative expression values were calculated using the ΔΔCt calculation with data normalized to the average of the geometric mean of 4 housekeeping miRNAs.

- Conclusion: Array-quantified miRNA can be obtained from D-FFPE bone marrow biopsy samples.

Additional references: