Isolation of High Quality RNA from Single Mammalian Cell

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Introduction

One of the major challenges of cellular molecular biology is the study at the single cell level. The study of genomics and transcriptomics at the single cell resolution is critical since most tissues, including normal and tumorous, are not homogeneous. Hence any expression analysis of a tissue sample may not reflect true expression profile of its constituent cells. In addition, developmental biology often involves the study of only a small population of cells including oocytes, stem cells and cells of early embryos. Three steps are involved in attaining single cell expression profiling – (1) Cell separation, (2) Nucleic acid purification, (3) Nucleic acid detection. In recent years, major advancements have been made in increasing the sensitivity in cell separation and detection. In particular, Laser-Captured Microdissection (LCM) and fluorescence-activated cell sorting (FACS) enables extraction of small population of tissue and single cell, respectively. Improvement in detection limit and new amplification technologies such as whole genome amplification allows the use of RT-qPCR, microarrays and RNA sequencing at single cell resolution. Interestingly, for RNA purification, only a handful of products are currently available, with the majority offering only cell lysate preparation. RNA isolated by these "Cell-to-Ct" products usually are in a chemical mixture that is optimized or limited for a particular downstream application or workflow. Here, we demonstrate the isolation of RNA from single cell and small cell population including LCM using Norgen's Total RNA Purification Micro Kit (#35300). The kit allows eluting of pure RNA in as little as 10 µL and in a simple solution without any detergent or chemical that interferes with downstream applications.

Methods

Single Cell Preparation

HeLa cells were initially quantified by a haemocytometer followed by end-point serial dilution down to one cell level per 5ul. Subsequently, ten 5ul droplets containing a single were dispensed in a 96well plate and the presence or absence of a single cell was confirmed by inverted microscope. RNA was isolated from the 5ul droplet with a single cell and from those with out any cell (as negative control).

RNA Isolation

RNA was isolated either using Norgen's Total RNA Purification Micro Kit or Arcturus PicoPure RNA Isolation Kit. In addition, six different LCM samples were obtained and isolated according to the specialized procedure described in the Norgen's kit.

RNA Detection and Quality Evaluation

RNA Isolated was used for RT-qPCR detection of various transcripts including S14 and S15 for large RNA and 5S rRNA for small RNA. In addition, RNA isolated from LCM samples was evaluated by NanoVue nanospectrophotometer.

Results

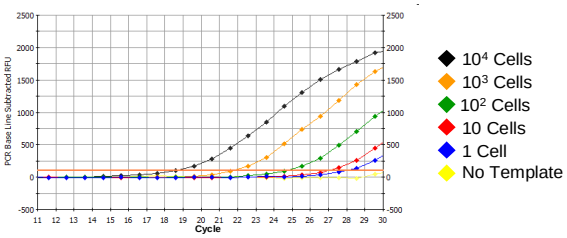

Figure 1. Great Isolation Sensitivity.

Total RNA was extracted from a decreasing number of HeLa cells followed by RT-qPCR to detect the human S14 transcript in the isolated RNA. PCR product of S14 was detected from a single cell.

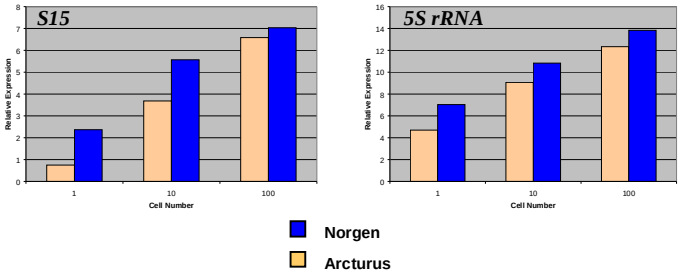

Figure 2. Better Efficiency of RNA Recovery at Single Cell Input.

Total RNA was extracted from a decreasing number of HeLa cells using Norgen's Total RNA Purification Micro Kit and a competitor's (Arcturus) spin column purification kit. The extracted RNA was subjected to RT-qPCR to detect the human S15 and 5S rRNA transcript in the isolated RNA. RNA isolated by Norgen's Total RNA Purification Micro Kit showed much higher relative expression of each RNA transcript tested at all cell input number down to single cell.

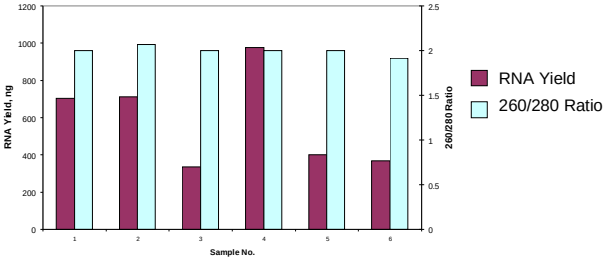

Figure 3. High Quality RNA Isolated from Laser-Captured Microdissection (LCM)

Norgen's Total RNA Purification Micro Kit allows sensitive but high quality RNA extraction from small input such as Laser-Captured Microdissection (LCM). Total RNA was isolated from 6 different LCM samples. RNA yield and quality (260/280 ratio) were assessed by NanoVue spectrophotometry. Norgen's Total RNA Purification Kit recovered good amount of RNA with excellent 260/280 ratio

Conclusion

Norgen's Total RNA Micro-Kit allows sensitive RNA purification from one cell.

Norgen's Total RNA Micro Kit outperforms competitor's kit (Arcturus).

Elution of Pure RNA in 10 µL of H2O, not in a form of lysate, that may interfere with down stream applications (eg. microarray or RNA sequencing).

Outstanding sensitivity of recovering both large and small RNA from one cell.

High quality RNA purified from a one cell and Laser-Captured Microdissection