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# A Systematic Comparison of Protocols for Recovery of High-Quality RNA from Human Islets Extracted by Laser Capture Microdissection

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**Abstract:** The isolation of high-quality RNA from endocrine pancreas sections represents a considerable challenge largely due to the high ribonuclease levels. Laser capture microdissection (LCM) of mammalian islets, in association with RNA extraction protocols, has emerged as a feasible approach to characterizing their genetic and proteomic profiles. However, a validated protocol to obtain high-quality RNA from LCM-derived human pancreas specimens that is appropriate for next-generation sequencing analysis is still lacking. In this study, we applied four methods (Picopure extraction kit, Qiazol protocol, Qiazol + Clean-up kit, and RNeasy Microkit + Carrier) to extract RNA from human islets obtained from both non-diabetic individuals and patients with type 2 diabetes who had undergone partial pancreatectomy, as well as handpicked islets from both non-diabetic and diabetic organ donors. The yield and purity of total RNA were determined by 260/280 absorbance using Nanodrop 100 and the RNA integrity number with a bioanalyzer. The results indicated that among the four methods, the RNeasy MicroKit + Carrier (Qiagen) provides the highest yield and purity.

**Keywords:** laser capture microdissection; RNA extraction; human islets



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## 1. Introduction

Laser capture microdissection (LCM) has emerged as a widely used technique for the isolation of specific types of cells or a minimum amount of parenchyma [1] for a variety of downstream analyses such as proteomic studies [2,3], RNA assays by microarray [4], or RNA sequencing [5,6]. This approach has been particularly useful to obtain small amounts of islet tissue to characterize the genetic profiles of both murine and human pancreas [7]. Compared to the analyses of handpicked islets, the use of LCM with specific staining for target cells avoids contamination by neighboring cells and the confounding effects of cell trauma/ischemia, which leads to alterations in cellular protein and gene expression due to the harsh chemical and/or mechanical processes during manual isolation. Indeed, previous reports [8], using handpicked islets, show an upregulation of pancreatic acinar and duct genes, suggesting contamination by non-endocrine cells, while the elevated expression of hypoxia- and apoptosis-related genes indicates changes secondary to mechanical effects. These findings were confirmed by Paraskevas et al. [9], who reported an upregulation of inflammatory markers, such as cytokines and cytokine receptors, in freshly isolated islets compared to beta cells from intact pancreas collected by LCM. Moreover, freshly isolated cells cultured for three days displayed a greater expression of transcription factors found in pancreatic progenitors, suggesting that islet isolation and culture together activate a process of de-differentiation of endocrine pancreatic cells. Thus, using chemical approaches to isolate pancreas islets could misrepresent the gene expression profile.

Given the limitations of manual islet isolation, the LCM approach is preferable to limit confounding results. Nevertheless, harvesting high-quality RNA from human islets that can be used for transcriptomic analysis is a continuing challenge, mostly due to the high level of intrinsic ribonuclease (RNase) activity in the pancreas [10,11]. One possible approach to minimizing the effect of RNase activity on the isolated cells and to increase RNA quality is the addition of a RNase inhibitor during both LCM and RNA extraction phases, as previously reported by Butler et al. [12]

Although the LCM technique [13] has improved, the quality and quantity of RNA collected from LCM human pancreatic samples is generally low, with no more than 100 ng of material after RNA amplification, compared to the 200–500 ng of RNA with at least an RNA integrity number (RIN) of 7, necessary to generate cDNA libraries for gene expression [14]. In this report, we compare the efficiency of different protocols used to extract RNA from human islet samples obtained by LCM in order to determine a better approach to ensuring RNA preservation that can be used by the scientific community for next-generation transcriptomic studies.

## 2. List of Equipment and Reagents

The following reagents were used:

- Tissue-Tek OCT medium (Sakura Finetek, Flemingweg, NL, USA, Cat# 4583)
- Isopentane (2-methylbutane) (Fisher Scientific, Waltham, MA, USA, Cat# 03551-4)
- DEPC-treated water (Invitrogen, Carlsbad, CA, USA, Cat# 750024)
- Ethanol 100% (Pharmco, Brookfield, CT, USA Cat# 1000200SG)
- Ethanol 70% (dilute 100% ethanol with DEPC-treated water to obtain 70% ethanol solution)
- Xylene (Fisher Scientific, Waltham, MA, USA, Cat# UN1307)
- SUPERase·IN (Ambion, Austin, TX, USA, Cat# AM2694)
- RNeasy Micro Kit 50 (Qiagen, Germantown, MD, USA, Cat# 740049)
- Qiazol lysis reagent, 50 mL (Qiagen, Germantown, MD, USA, Cat#55402828)
- RNeasyMinElute Clean-up Kit 50 (Qiagen, Germantown, MD, USA, Cat#74204)
- PicoPure RNA isolation kit (Applied Biosystems by Thermo Fisher Scientific, Vilnius, Lithuania, Cat# KIT0204)
- RNase-Free DNase Set 50 (Qiagen, Germantown, MD, USA, Cat# 79254)

The following laboratory materials and equipment were required:

- Cryomold (Fisher Scientific, Waltham, MA, USA, Cat# 22-038217)
- Frosted microscope slides (Corning, New York, NY, USA, Cat# 2948-75X25)
- Polypropylene Falcon Tube (Fisher Scientific, Waltham, MA, USA, Cat# 14-959-49A)
- RNaseZap, 250 mL (Ambion, Austin, TX, USA, Cat# 9780)
- CapSure HS LCM Caps (Arcturus Engineering, Mountain View, CA, Cat# LCM0214)
- GeneAmp® Autoclaved Thin-Walled Reaction Tubes (Applied Biosystems by Thermo Fisher Scientific, Vilnius, Lithuania, Cat# N801-0611)
- Tweezers and forceps
- Pipettes: 20–200 µL and nuclease-free pipette tips
- Cryostat
- Fume hood
- PixCell® IIe Laser Capture Microdissection System (Arcturus Engineering, Mountain View, CA, USA)
- Incubator (Fisher Scientific, Waltham, MA, USA, Cat# 11690506D)
- Microcentrifuge (Fisher Scientific, Waltham, MA, USA, Cat# 05-090-128)

## 3. Materials and Methods

To compare the efficiency of different RNA extraction protocols in frozen human islet samples, we used LCM to collect a mean of 100 islets from pancreatic surgical specimens, obtained from non-diabetic or diabetic patients who had undergone partial pancreatectomy for an extra-pancreatic tumor. Surgical procedures were performed by the Hepato-Biliary