

Characterizing Gene Expression in nPOD Donor Islets

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Purpose: The purpose of our study is to use laser-capture microscopy to collect islets from auto-antibody positive and control (ab negative) nPOD donors, and obtain a comprehensive islet mRNA expression profile. Comparing the two groups will allow us to gain insights into affected pathways and molecular abnormalities in islets from people in prediabetic and early diabetic stages.

Methods: We prepare slides to be RNase free and send them to the nPOD laboratory where pancreatic tissue cryo-sections are cut onto the slides, then shipped back to us on dry ice. Slides are fixed, dried, and then laser-capture microscopy is conducted to capture 60-80 islets from each sample. RNA is immediately extracted from the tissue and stored at -80C. The quality and integrity of total RNA is tested using an Agilent Bioanalyzer 2100 system and quantified by Nano-Drop 2000. Only samples with intact ribosomal RNA peaks (18s, 28s), 260:280 absorbance ratios of 1.8-2.1, and RNA Integrity Numbers (RIN) of above 5.0 are processed further. The RNA is amplified using kits that are appropriate for the downstream transcriptome or RT-PCR experiments. Both Affymetrix expression arrays and RNA sequencing technologies are employed to obtain global gene expression signatures from autoantibody positive and negative tissue donors.

Summary of Results: We have developed methods and procedures that allows us to obtain high quality RNA from some islet donors (RIN numbers of 5.6-7.3, n=4). However from other donors the RNA was of much less quality (RIN numbers of 2.2-4.5, n=8).

Conclusions: We have demonstrated the feasibility of obtaining comprehensive gene expression signatures from islets from approximately 1/3 of the nPOD donor pancreata using laser-capture. Gene expression studies in most nPOD donor islets may require use of methods that are less sensitive to the quality of the RNA.