CRYOXTRACT
An Allied Minds Company

CXT 750
FROZEN SAMPLE ALIQUOTTER

APPLICATION NOTE

Evaluation of Frozen Coring and RNA Quality

Using CryoXtract's CXT 750 Frozen Sample Aliquotter

Introduction

RNA is a frequently utilized and studied bio-molecule in modern translational medicine, drug development, and broader scientific efforts. However, the highly sensitive nature of RNA that results from its single strand structure and susceptibility to enzymatic degradation can create significant challenges when working with it. As a result, valuable samples of RNA are often stored in frozen state and are fully consumed when accessed in order to avoid undesired multiple freeze-thaw cycles. The CXT 750 Frozen Aliquotter provides a novel method for the repeated access and aliquotting of larger volume frozen bio-samples such as RNA.

Experimental Overview

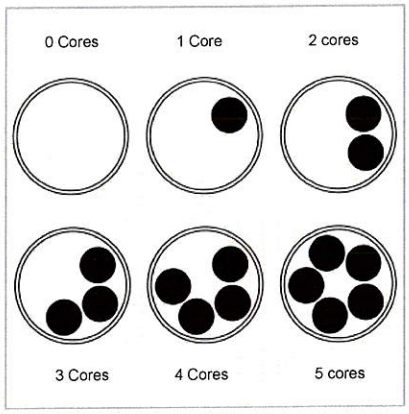
In an independent study, frozen aliquotting was evaluated for its potential utility in processing RNA-based biosamples. The primary objective of the study was to determine if the physical stresses of frozen aliquotting would impact the quality of RNA exclusive of enzymatic degradation.

To this purpose, RNA was isolated from cell pellets and eluted into RNase/DNase-free water. Isolated RNA from multiple cell pellet isolations were then pooled to provide sufficient volumes of RNA for this study. In total, 4 pooled stocks of RNA were generated and designated as RNA A, RNA B, RNA C, and RNA D. Each stock was analyzed on an Agilent Bioanalyzer 2100 in order to establish a baseline RNA quality profile.

One (1) ml of each stock was then aliquotted into 1.8ml crovials, frozen and stored at -80^{0}C in preparation for processing on the CXT 750 Frozen Sample Aliquotter. The remainder of each fresh RNA stock was also frozen and stored at -80^{0}C in order to evaluate frozen RNA samples not subjected to frozen aliquotting.

The samples for frozen aliquotting were cored using the CXT 750 Frozen Sample Aliquotter in a single session that produced 5 cores per vial of RNA stock. On the CXT 750 Frozen Sample Aliquotter, each sample is processed for the number of cores designated by the user. The instrument maintains both the parent samples and frozen aliquots at -80C throughout the processing and includes an integrated automated coring probe cleaning to eliminate potential carryover contamination between samples.. Figure 1 illustrates the coring pattern for a sample scheduled for 5 aliquots, as was in the case of this study. Coring proceeds in a clockwise and pentagonal pattern. Frozen cores obtained from the coring of these samples were stored at -80^{0}C prior to performing RNA quality analysis.. Core 1 RNA samples (RNA A, B, C, and D) were measured 22 days after the baseline reading. Cores 2, 3, 4, and 5 samples were measured at 23, 24, 25, and 26 days, respectively.

Figure 1: CXT 750 Frozen Sample Aliquotter Coring Pattern



The remainders of the fresh RNA stocks, which also had been frozen and stored at -80C, were thawed and measured for RNA quality and served as a non-cored frozen controls ("Control").

Results

Figure 2 presents the resulting electropherograms from an Agilent Bioanalyzer 2100 for samples tested from the RNA A stock. Each electropherogram is labeled according to the 3 condition tested: Baseline, Control, and Cores 1 through 5.

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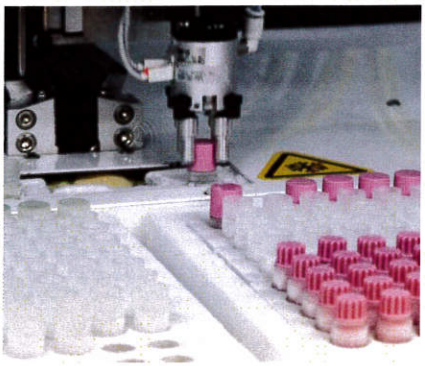
APPLICATION NOTE

Utilization of Frozen Aliquotting in Genomic Applications and Workflows

Using CryoXtract's CXT 750 Frozen Sample Aliquotter



Introduction

DNA is perhaps the most significant and enabling bio-molecule in medical and scientific research today. The marriage of genomic science with translational and diagnostic medicine has led to vast stores of bio-fluids in which DNA can be isolated for a variety of vital medical applications and scientific research. Automated and systematic access to such samples is recognized as a growing and critical requirement for facilities and laboratories supporting these efforts.

Experimental Overview

An independent study conducted in collaboration with a modern, CAP-accredited bio-bank evaluated the processing of gDNA utilizingCryoXtract's properietary CXT 750 Frozen Sample Aliquotter . The CXT 750 Frozen Sample Aliquotter was deployed in 2 different but common DNA workflows. First, DNA was purified from a single, expanded lymphoblastoid cell line (LCL DNA) and rehydrated with a TE buffer and aliquotted into twenty 2mL aliquots in standard 1.8mL cryovials. Each sample was assayed using a proprietary SNP (single nucleotide polymorphism) panel designed as a functional quality control (QC) measure of DNA integrity. All samples were then frozen at -80°C. Five frozen cores were isolated from each LCL-DNA sample in a single run per group using the CXT 750. LCL-DNA Cores and residual were immediately stored at -80°C and frozen cores were later re-assayed using the proprietary SNP panel.

Second, previously frozen EDTA whole blood was thawed and aliquotted into 23 standard 1.8mL cryovials at 2mL each and then re-frozen at -80°C. Frozen cores were obtained from 14 of these samples using the CXT 750 Frozen Sample Aliquotter, The remaining 9 samples were used for other elements of this study not reported on in this note. DNA was extracted from whole blood (FWB-DNA) using the QIAsymphony DNA mini kit and DNA Blood 200 protocol (Qiagen). The FWB-DNA extracted from frozen whole blood cores was subjected to QC measures using the proprietary SNP panel to determine functional performance.

Table 1: SNP Panel Results[1]

DNA Source	Number of Passes	Number of Failures	Pass Rate
LCL-DNA	93	2	97.9%
FWB-DNA	70	1	98.6%

[1]The proprietary SNP panel used in this study was designed specifically for the QC of DNA through a strategic selection of highly polymorphic SNPs (single nucleotide polymorphisms) capable of characterizing a DNA sample in relation to depredation events as well as a host of other genetic characteristics (gender, parentage,

Results

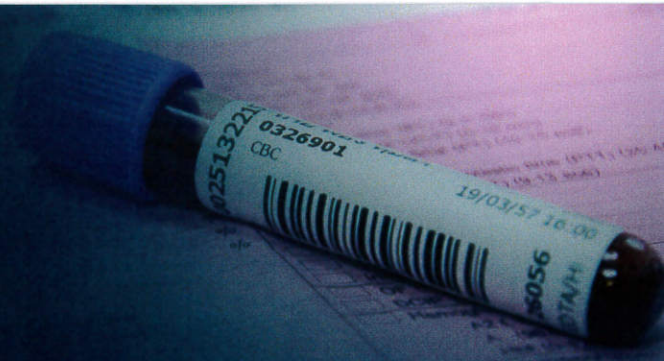
The reported metric of this QC procedure is a simple pass/fail indicator. For LCL-DNA, a total of 95 cores were assayed on the SNP panel resulting in 2 failures and 93 passes. This equates to a 97.9% pass rate for LCL-DNA processed by frozen aliquotting. For whole blood samples processed on the CXT 750 Frozen Sample Aliquotter, Seventy-one samples of FWB-DNA extracted from 71 cores were assayed resulting in a pass rate of 98.6% (1 failure, 70 passes). Investigators performing the work found these results to fall well with performance expectations.

Conclusions

Two commonly utilized sources of DNA (isolated from cell lines and suspended in TE buffer and frozen whole blood) were demonstrated to be compatible with frozen coring technology. LCL-DNA and FWB-DNA were both shown to be functionally intact for down stream applications when assayed using a proprietary SNP panel.

Clinical Trials

Frozen Aliquotting and Sample Quality Control



"We have a number of outliers in our biomarker data sets. Can we determine if outliers are related to sample handling / pre-analytical events?"

"I would like to utilize retrospective samples for an upcoming study. Is there a way to verify that sample quality is acceptable for testing?"

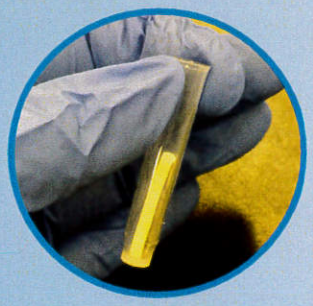
"We receive samples from several collection sites. Can we verify that individual sites are following the SOP for sample processing?"

You can with the MxP® QC Plasma.

Metabolomic Profiling for Quality Control

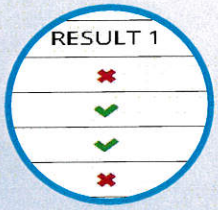
The MxP® Quality Control Plasma is a metabolomics-based profiling assay which provides holistic quality control of human EDTA plasma samples. Proprietary algorithms evaluate results for unknown samples against known profiles. Results provide information about the presence of pre-analytical deviations and where they occur in the process.

Frozen aliquots enable evaluation of sample quality and preservation of parent samples. Retrospective samples can be tested to determine the quality of a sample set. Samples from a specfic collection site can be tested to ensure compliance with SOPs. Poor quality outliers can be removed to increase data accuracy and improve trial outcomes.



1 COLLECT SAMPLE
Core Frozen Aliquot

2 TEST SAMPLE
Perform GC-MS Analysis



3 EVALUATE SAMPLE
Choose Based on Quality Report

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