nRichDX Revolution™ instrument and cfDNA Isolation Kit for extraction of cfDNA from Large Plasma and Urine Sample Volumes Improves Yield of Rare Targets

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INTRODUCTION

Precision medicine requires the ability to detect circulating tumor DNA (ctDNA) and to monitor the evolution of mutations during treatment. Detecting ctDNA in plasma and urine is challenging because it constitutes only a minor fraction of the total cell-free DNA (cfDNA). Most commercially available cfDNA isolation kits are limited to small input sample volumes (0.1mL- 5mL), making the detection of rare targets difficult. The nRich^{DX} Revolution System and cfDNA Isolation Kit are capable of extracting 3mL – 20mL of plasma or urine by combining extraction, enrichment, and concentration of cfDNA without the need for transfer steps. Here we compare the nRich^{DX} Revolution System and cfDNA Isolation Kit extraction efficiency, cfDNA quality, and yield to the MagMAX™ cfDNA Isolation Kit. (NOTE: The Poster Abstract has been revised since the submission.)

MATERIAL AND METHODS

Normal human plasma donor pools and urine samples were extracted with either the nRich^{DX} Revolution cfDNA Isolation Kit or the MagMAX cfDNA Isolation Kit. Each sample was spiked with varying amounts of a BRAF mutant fragment (220bp). The total yield was quantified (Denovix DS-11) and the quality of cfDNA was determined by analysis on the Agilent TapeStation. Efficiency of extraction of mutant BRAF was determined by qPCR. The ability of the nRich^{DX} product to extract larger sample volumes of plasma and urine was also determined. 3mL – 20mL of plasma or 10mL – 20mL of urine were each spiked with varying amounts of a BRAF mutant fragment. In addition, urine samples were spiked with a DNA base pair ladder control. The yield, quality, and efficiency of cfDNA were determined as noted.

Figure 1: nRich^{DX} Revolution™ instrument

Study 1: Cross platform evaluation. Compare nRich^{DX} vs MagMAX extraction protocols with 5mL plasma. Assessment of DNA concentration yield, quality, and efficiency.

Study 2: Evaluation of extraction from different starting input volumes of 3ml - 20ml of plasma or 10mL - 20mL of urine. Assessment of DNA concentration yield, quality, and efficiency.

RESULTS

Study1:
Four aliquots of 5mL plasma from 4 plasma donor pools spiked with a BRAF mutant fragment were extracted for cfDNA and averaged 92ng for nRich^{DX} and 70.8ng for MagMAX (Figure 2). qPCR mutant BRAF detection was confirmed by both methods with an input of 35cp/mL.

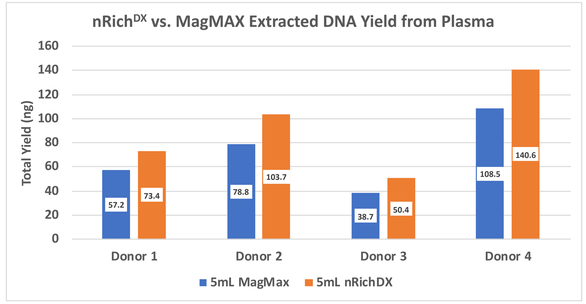

Figure 2: cfDNA yield extracted from 5mL donor plasma with nRich^{DX} or MagMAX

The cfDNA was then assessed for purity and quality using the Agilent TapeStation (Figure 3). The spectrograph traces show similar patterns with a large fragment peak size of 185bp for nRich^{DX} and 189bp for MagMAX, the expected sizes of cfDNA. The two extraction methods appear comparable in performance in terms of quality of cfDNA product.

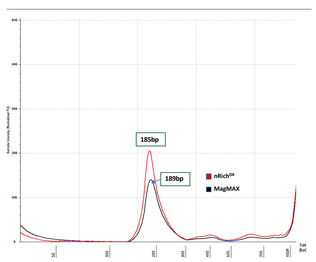

Figure 3: Spectrograph of cfDNA extracted from 5mL plasma with nRich^{DX} or MagMAX

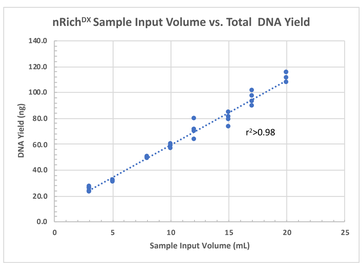

Figure 4. cfDNA yield extracted from 3ml - 20ml of donor plasma with nRich^{DX}

Study 2:
Aliquots of plasma from 3mL to 20mL donor plasma were extracted using the nRich^{DX} cfDNA Isolation Kit. There was a proportional increase in cfDNA yield as volume increased (r^2>0.98). The increased volume of 15mL plasma resulted in an extracted product enriched in the target cfDNA (150-225bp), however an increase of larger-sized fragments (>300bp) was also seen (Figure 5). The limit of detection of BRAF mutant improved as the plasma volume increased. All 15 mL extractions containing 12cp/mL of mutant BRAF detection were detected by qPCR; an input of 35cp/mL was required for 100% detection of BRAF mutation with a 5mL extraction.

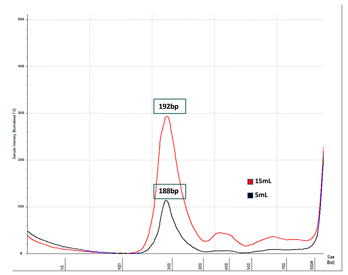

Figure 5: Spectrograph of cfDNA extracted from 5mL and 15mL plasma with nRich^{DX}

Aliquots of 10mL donor urine extracted with MagMAX gave an average of 30.4ng compared to 65.8ng with nRich^{DX}. The average yield of a 20mL extraction with nRich^{DX} was 96.4ng (Figure 6). There was excellent recovery by the nRich^{DX} cfDNA Isolation Kit of a 500ng spike in 20mL urine spiked with the Thermo Fisher Low Range DNA Ladder (75bp - 700bp) as shown in Figure 7.

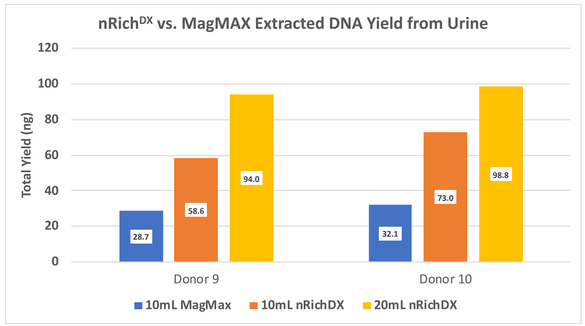

Figure 6. cfDNA yield extracted from 10mL and 20mL of donor urine with MagMAX or nRich^{DX}

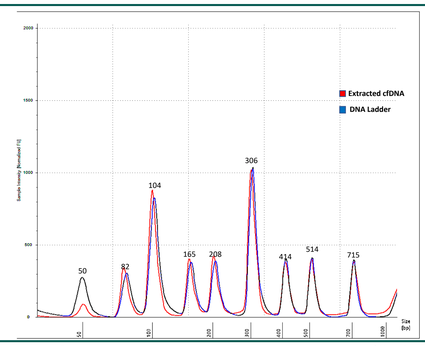

Figure 7. Spectrograph of DNA ladder spike in urine with nRich^{DX} cfDNA Isolation Kit

CONCLUSIONS

The nRich^{DX} product markedly improves cfDNA extraction by allowing a greater input volume of plasma or urine which enhances detection of rare targets. The nRich^{DX} system delivered larger yields of total cfDNA compared to the MagMAX kit at equivalent quality.