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Utilising CAPTURE-XT®, a rapid pathogen concentration and sample clean-up technology, as a front-end for Next-Generation Sequencing (NGS) of *Mycobacterium tuberculosis* from clinical sputum samples

D. MCGURK[1], G. MORGAN[1], J. CAFFRY[1], H. MURTON[1], M. SELBY[1], P. SCULLY[1], M. PARTINGTON[1], C. MORAIS[1], K. GIZYNSKI[1], J. O'HALLORAN[1]

1 QuantumMDx, Newcastle Upon Tyne, United Kingdom

QuantuMDx THE FUTURE

CAPTURE-XT® PATHOGEN CONCENTRATION

INTRODUCTION

The application of Next-Generation Sequencing (NGS) for the genetic analysis of *Mycobacterium tuberculosis* (MTB) direct from sputum faces a number of problems as a result of the complex sample matrix.

Sputum contains a high concentration of DNA from the human host and non-target commensal organisms which results in wasted reads in NGS[1]. This is typically overcome by culturing the samples, but culture drastically increases turnaround times and is susceptible to contamination.

The application of a technology capable of rapidly and selectively enriching target cells from sputum could make NGS much more accessible for researchers in the future.

AIM

The aim of this project was to explore the application of CAPTURE-XT®, a microfluidic lab-on-chip pathogen concentration system which utilises dielectrophoresis (See Figure 2) to selectively capture and concentrate whole MTB cells from sputum, as a potential front-end for NGS.

METHOD

Sample Preparation

1. MTB (10^6 CFU/mL) in a proprietary DEP-compatible buffer (GP Buffer)
2. MTB (10^6 CFU/mL) & Human tissue culture (CaSki, 10^6 cells/mL)
3. MTB (10^6 CFU/mL) in clinically negative sputum (provided by SGUL)
4. Clinically positive sputum (smear-positive 3+; provided by UCT)
5. Clinically positive sputum (smear-negative, culture positive; provided by UCT)

Sample Processing

Samples were split and were processed by either through the CAPTURE-XT® system, or by a typical NALC/NaOH sedimentation methodology[2].

Sample lysis and clean-up

The products from each processing methodology were lysed by bead-beating and enriched using AMPure-XP beads before being eluted into Tris-EDTA buffer for library preparation and NGS.

NextSeq Whole Genome Illumina Sequencing

Illumina sequencing was performed by the Genomic Core Facility in Newcastle University. Multiple-sequence alignment was performed against a reference MTB genome using the Burrows-Wheeler Algorithm.

RESULTS

Sample Enumeration

Following clean-up, the concentration of target DNA was enumerated by qPCR (Fig. 1A) and the total DNA concentration was enumerated by Qubit (Fig. 1B).

Figure 1A shows that both sample processing methodologies were successful, yielding a similar final concentration in most samples, with the most notable difference being the poor performance of sedimentation in buffer. It is likely that the NALC/NaOH combination caused damage to the DNA in this sample.

Figure 1B shows that sedimentation typically exhibited a higher **total DNA** yield which, when combined with the evidence that both workflows offered similar yields of MTB DNA, indicates that sedimentation yielded a high concentration of non-target DNA. A notable exception to this being in the clinical smear negative sample, where CAPTURE-XT® exhibited a low target-DNA and a high total DNA yield, indicating some non-specific capture.

Samples were diluted to normalise the **total DNA concentration** prior to sequencing.

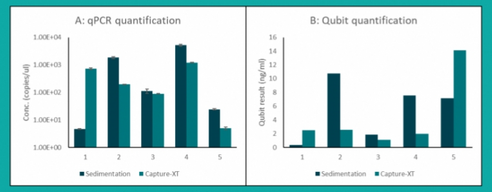

Figure 1 - Enumeration of samples. After samples were processed by either sedimentation or CAPTURE-XT®, DNA yields were enumerated by qPCR (A) and Qubit (B). 1: MTB in GP buffer; 2: MTB & CaSki cells in buffer; 3: MTB in clinically negative sputum; 4: Clinically positive (smear 3+); 5: Clinically positive (smear -ive, culture +ive)

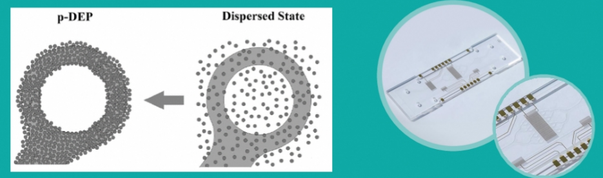

Figure 2 - Visual representation of Dielectrophoresis. When an alternating current is applied to the electrode, particles experience an attractive force (pDEP). A particle can be specifically targeted based on physical properties, such as size, by altering electrode dimensions or electrical properties, such as signal frequency.
The design of a CAPTURE-XT® cassette can be seen on the right, microfluidic channels containing a biological sample pass over interdigitated electrodes which are tuned to specifically capture MTB, the cells can then be eluted into a clean buffer for downstream analysis.

Multiple-sequence Alignment

Table 1 shows that all samples processed by CAPTURE-XT® exhibited a higher coverage of the reference genome than those which were processed using by sedimentation, with a substantial improvement in performance for samples in buffer and Clinical 3+ samples.

High performance in buffer was expected due to the sole presence of target-DNA in the sample; it is likely that the poor performance of sedimentation in this sample was due to DNA damage caused by the NALC/NaOH treatment, which was also evident in figure 1.

The high performance of CAPTURE-XT® in clinical smear 3+ sample shows great potential for the system, offering a potential improvement over traditional methods which experience poor performance due to the high concentration of non-target DNA in the sample.

It is notable that clinically negative sputum spiked with MTB performed poorly with both methodologies, although these samples were MTB-negative, they were likely produced by patients with a clinically relevant concentration of non-MTB respiratory pathogens which may have resulted in many wasted reads during NGS.

Poor performance of both methodologies in clinical smear negative, culture positive samples was expected due to the extremely low concentration of MTB.

Table 1 - Genome coverage of each sample against a reference genome. Multiple-sequence alignment (Burrows-Wheeler) of the NGS data was performed for each sample against a reference H37Ra genome.

Sample Description	Alignment against *M. tuberculosis* H37Ra genome (%)	
	Sedimentation	CAPTURE-XT®
MTB in buffer	2.46%	62.10%
MTB + CaSki in buffer	5.63%	9.21%
MTB in negative sputum	0.14%	0.15%
Clinical Smear 3+	0.71%	61.39%
Clinical smear negative, culture positive	0.10%	0.16%

CONCLUSIONS

The overall performance of CAPTURE-XT® in these experiments showed great promise as a potential front-end for NGS, particularly considering the high performance in clinical 3+ samples.

As part of this proof-of-concept study, some areas requiring improvement were highlighted, but the overall performance increase with respect to the typical sedimentation method shows potential, even in the more poorly performing sample types.

The performance of CAPTURE-XT ® as a front-end to NGS could reduce costs associated with routinely performing NGS by reducing the number of reads required to obtain a high quality sequence compared to sedimentation and reducing the turnaround time from sample to result required by MGIT culture. Making NGS of MTB more accessible could lead to significant improvements in susceptibility testing, epidemiological study, and diagnostics.

REFERENCES

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CONTACT INFORMATION

If you have any questions, please don't hesitate to contact us:

Telephone: +44 (0) 870 803 1234

Email: info@quantumdx.com **Website:** www.quantumdx.com