IMPROVED METHOD FOR NGS LIBRARY PREPARATION FROM FFPE SAMPLES

Key benefits

- NGS DNA library preparation method suitable for Whole Genome and Targeted Genome Sequencing
- Higher DNA coverage and increased library yield from low input and badly degraded samples
- Suitable for formalin-fixed paraffin-embedded (FFPE) samples (2 ng for grade 1 to 10 ng for grade 3 FFPE samples)
- Decreased method-induced bias due to less amplifying errors (fewer PCR cycles needed)
- Per sample cost is lower than commercially available kits

Background

The desire to analyse limited amounts of biological material, historic samples, and rare cell populations has collectively driven the need for efficient methods for whole genome sequencing (WGS) of limited amounts of poor quality DNA. Most protocols are designed to recover double stranded DNA (dsDNA) by ligating sequencing adaptors to dsDNA with or without subsequent PCR amplification of the library. While this is sufficient for many applications, NGS library preparation from limited, highly degraded DNA from FFPE samples is challenging with current kits. We have developed a method that has potential to unlock WGS data from DNA previously considered impossible to sequence, broadening opportunities to understand the role of genetics in health and disease.

The Problem

- Retrospective studies require FFPE tissue preservation so there is a need for a new methodology to improve sequencing quality
- Standard library preparation protocols require an adaptor ligation step, which is inefficient and only recovers dsDNA
- WGS of fragmented genomic DNA is associated with sequencing and mapping artefacts, which are significantly more prevalent in FFPE samples
- FFPE treatment can result in extensive DNA damage (particularly DNA crosslinks and deamination of cytosines), leading to poor quality sequencing data rendering many samples unusable for WGS and fragmentation

The invention *vs NEB Next Ultra II (E76455/ M6630S)*

- Our method using as little as **2 ng of poor quality FFPE DNA**, results in a 2.5 fold increase in sequence coverage. 80% of these reads have a high mapping quality (MAPQ >= 20) compared to 70% using the standard method.
- Sequencing of **substantially degraded FFPE samples** (10 ng input) produces 23 fold higher yield, higher median insert sizes, indicating better library quality and genome coverage is up to 3.77 fold higher compared to the standard method.
- In a head-to-head comparison, our method identified a putative driver mutation in human cancer that was not identified using the standard method. The data were likely filtered out due to the minimum number of reads covering the base in the standard method.

Link to inventor website: https://www.bci.qmul.ac.uk/en/staff/item/gabriella-ficz

Project Development and Results

We are currently seeking for partners to bring the technology to the market.

Queen Mary University of London

Queen Mary Innovation
qminnovation.co.uk
e.ledaki@qmul.ac.uk
+44 (0)7920 270995