DNA Extraction Methodologies for Ultra Long Nanopore Sequencing to Determine Chromosome Specific Rodent Telomere Lengths

Kidd, E.[1], Mooruth R[1], Meimaridou, E.[1], Walley, A.J.[2] and Fairbrother, UL.[1*]

[1] *School of Human Sciences, London Metropolitan University, London, N7 8DB, UK*

[2] *Section of Molecular Biology, Institute of Medical and Biomedical Education, St George's, University of London, London, SW17 0RE, UK*

Telomeres are involved in gene expression through Telomere Position Effect Over Long Distance (TPE-OLD) and indirectly through telomeric repeat containing RNA (TERRA). It is important to fully elucidate relationships between TL and disease, by observing TL, not only on average, per tissue or per cell, but of specific chromosome ends. Ultra long sequencing has been attempted with Human TL (5-15Kb) with some success. However, animal model TL, such as rat and mouse, are longer (20-100kb, and 30-150kb, respectively) and more problematic.

Previous work in our lab shows that phenol/chloroform extraction damages DNA less than spin columns and magnetic beads and that extraction methods are crucial for accurate TL determination. We have generated long reads using low impact DNA extraction methods and nanopore sequencing with a Mk1C . Rat DNA was isolated using isotachophoresis (Purigen) and a mouse DNA sample isolated using phenol/chloroform. We designed a system of tagging telomere ends with a custom probe that hybridises to the 3' overhang present at the end of telomeric DNA, to ensure we are capturing the entire telomere.

We generated sequence of similar ultra long reads of 120-170kb for both mouse and rat samples extracted with isotachophoresis and phenol/chloroform methodologies respectively. We have generated putative telomeric/subtelomeric reads of < 455Kb using an adaptive sampling approach, focussing on mouse chromosome 1.

Direct sequencing of highly repetitive stretches of DNA is difficult, using specific DNA extraction techniques and adaptive sampling and tagging approaches for targeted enrichment has generated chromosome specific ultralong reads in rodents.

1. Kidd et al., 2023