External validation of Fire Monkey

Complete genome assemblies of rare Salmonella enterica serovars using ONT & Illumina sequencing

August 2020

This publication describes the work of two groups (Canadian Food Inspection Agency & Gastrointestinal Bacteria Reference Unit, Public Health England). These two government agencies from Canada and the UK used the Fire Monkey kit to extract DNA from 12 Salmonella enterica serovars and ONT plus Illumina reads to achieve complete genome assembly for all isolates.

Reference:

Gao R *et al* (2020) Complete Genome Assemblies of the Rare Salmonella enterica Serovar Adjame Using Nanopore and Illumina Sequence Reads. Microbiology Resource Announcements. Volume: 9, Issue: 35. e00280-20

Fire Monkey DNA/RNA co-extraction for nucleic acid ratio determination

June 2020

RNA assays measure the expression and therefore activity of a certain gene, however because these expression levels can vary between different samples it is important for the expression levels to be normalized. SpeedX is an Australian-based company that specializes in infectious disease diagnostics and has developed a multiplexed PCR assay that utilizes DNA to normalize RNA expression values. For this reason, and in order to reduce sample variability, it is important that DNA and RNA are extracted simultaneously. In one of their patent applications Fire Monkey version 1 was used to co-extract DNA and RNA and that was used to purify nucleic acid from both pure bacterial culture and clinical urine specimens, followed by multiplexed-PCR analysis.

Reference:

Todd AV & NE Lima (2020) NUCLEIC ACID RATIO DETERMINATION (Patent Application). Pub. No.: US 2020/0199651, Pub. Date: Jun. 25, 2020

Foodborne Salmonella enterica drug resistance

September 2020

This publication describes the work of 3 groups (Quadram Institute, Guangdong Laboratory for Lingnan Modern Agriculture in Guangzhou, China & Norwich Medical School, UEA) on identifying mutations related to ciprofloxacin heteroresistance. Heteroresistance means that a population of bacteria have different levels of sensitivity to a given antibiotic. Antibiotic exposure can increase the proportion of resistant mutants which could lead to full resistance. Therefore, it is important to be able and identify the emergence of heteroresistance. The Fire Monkey kit was used to extract DNA from Salmonella enterica isolated from chicken meat, and ONT sequencing analysis was performed.

Reference:

Zhang CZ *et a* (2020). Emergence of ciprofloxacin heteroresistance in foodborne Salmonella enterica serovar Agona. Journal of Antimicrobial Chemotherapy. doi: 10.1093/jac/dkaa288

High Quality DNA Extraction for Long Read Sequencing

Simple user friendly protocol. Less hands on time

"Getting good quality genomic DNA with high molecular weight with longer length is essential to get good quality sequencing reads from long read sequencing platform. Using Revolugen's Fire Monkey HMW DNA extraction kit, greater than 100 kb genomic DNA were extracted from bacterial samples, which indeed yielded high quality long read sequencing data from Nanopore sequencing. N50 of contigs from such DNA were 25-30 Kb length."

Overall	Quality of Results	Ease-of-Optimisation
★★★★★	★★★★★	★★★★★

The Bottom Line:

"Highly recommended kit for high quality high molecular weight DNA for long read and other types of sequencing. Other available kits in the market are nowhere near to this Revolugen kit."

Harvard University 10X Genomics & Illumina technology validation

June 2020

This Harvard group used Fire Monkey to extract samples for sequencing with the 10X Genomics-Illumina workflow to identify which genes were positioned on each parental genetic contribution. Being positioned on the same chromosome is important for dominance expression and interactions. This Haplotype phasing is of major structural importance to determine complete chromosomal haplotypes of diploid human genomes. It demonstrates and validates the use of Fire Monkey with the 10x Genomic and Illumina technologies.

Reference:

Tourdot & Zhang (2020) Complete Haplotype Determination and Single-Chromosome Analysis.

The Quadram Institute Illumina technology validation

February 2020

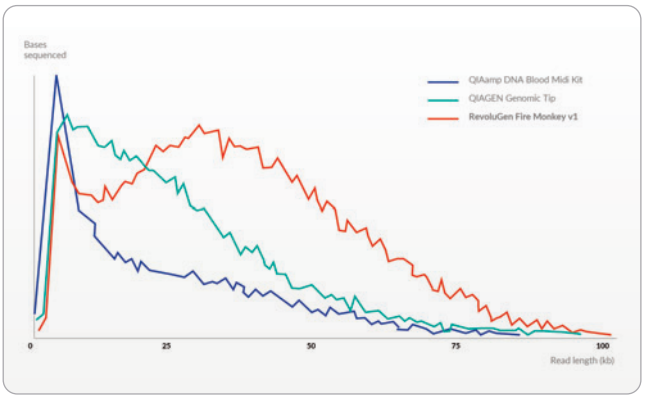
Extensively drug-resistant (XDR) Salmonella Typhi causes Typhoid fever that is endemic in Pakistan and shows widespread resistance to first line drugs such as ampicillin, co-trimoxazole, chloramphenicol and fluoroquinoles. XDR isolates from patients in Pakistan were processed using the Fire Monkey protocol and whole genome sequencing using Illumina technology was performed at the Quadram Institute. These strains were found to harbor the same resistance determinants as the outbreak strain.

Reference:

Rasheed *et al* (2020) Emergence of Resistance to Fluoroquinolones and Third-generation Cephalosporins in Salmonella Typhi in Lahore, Pakistan. medRxiv Chromosome Analysis.

Oxford Nanopore Technologies sequencing technology validation

Oxford Nanopore technologies has published the successful use of Fire Monkey DNA extraction with its sequencing technology on its own website. ONT validated an early version of Fire Monkey (v1) and revealed that Fire Monkey produced significantly higher N50 values than Qiagen's Genomic Tip product (25-35kb vs 20-30kb) in a fraction of the time. The following results



RevoluGen Fire Monkey v1	
Difficulty	Easy / Medium
Time Taken	1 hour
Input	1ml whole blood
Extraction Yield	8µg
Read N50	25–35kb

QIAGEN Genomic Tip	
Difficulty	Easy / Medium
Time Taken	4-6 hours
Input	10ml whole blood
Extraction Yield	75µg
Read N50	20–30kb

QIAamp DNA Blood Midi Kit	
Difficulty	Easy / Medium
Time Taken	1.5 hours
Input	1ml whole blood
Extraction Yield	12µg
Read N50	10–15kb