REVOLUGEN

FIRE MONKEY

# Fire Monkey: Spin-column kit for both Short and Long read sequencing

FEBRUARY 2023


Fire Monkey is a spin column based Nucleic Acid Isolation and Purification (NAIP) kit that performs High Molecular Weight DNA (HMW-DNA) extraction & simultaneous size selection under the Fire Monkey protocol for bacteria and animal cells. Fire Monkey takes about one hour to perform and results in average DNA strand lengths of appx. 100kb and more (Femto Pulse analysis).

The Fire Monkey protocols have the proprietary and unexpected ability to capture the long DNA fragments without breaking them too much whilst also depleting the short DNA fragments, despite high force spinning. This is the major ground-breaking innovation of this kit which makes it the world's first spin column HMW--DNA NAIP.

From sample to DNA in **1h**

**Spin column**
Standard format

**> 100kb**
Average extract size

**FIRE MONKEY HMW-DNA Extraction kit**

Multiplexing **48x** bacterial isolates

**10-20kb**
Size selection cut-off

Mammalian cells throughput up to **160 Gb**

## Saves work and money

Fire Monkey extraction avoids a size selection step and its output is immediately ready for library prep

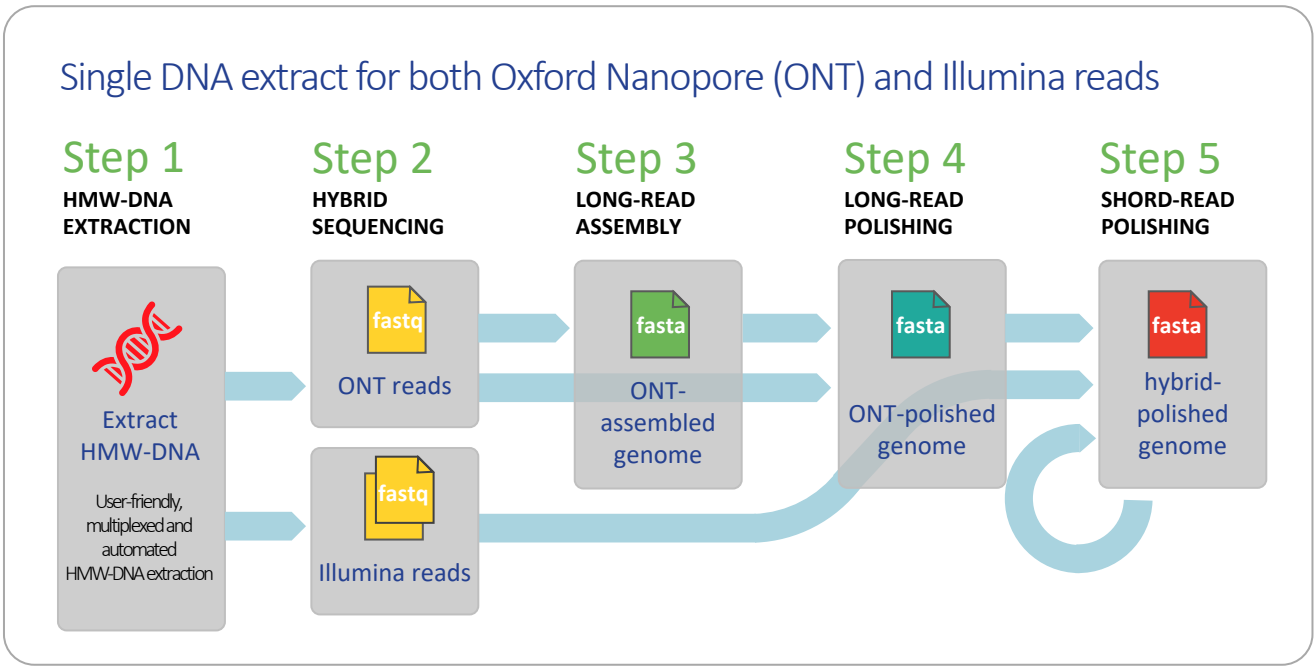
Fire Monkey extracted DNA significantly reduces the sequencing cost in $/bacterial genome terms.

## Cheaper sequencing

Big yield of Not too short/Not too long DNA cuts $/bacterial genome costs

# Hybrid assemblies

Because Fire Monkey is as user-friendly as any spin column kit it gives Illumina sequencing laboratories the option, at little extra cost, to keep the same sample extract for both their initial short read sequencing and for any subsequent long read sequencing they may decide to perform at some point in the future. It also provides a minimally disruptive entry point into long read sequencing as it uses a spin column protocol that everyone is already familiar with. If Fire Monkey is adopted as the standard extraction protocol for their short read sequencing needs, any long read sequencing can therefore be performed on exactly the same original sample that the Fire Monkey kit extracted without any further cost, reprocessing problems or potential handling errors.




**Adapted from:**
Wick *et al* (2022) Assembling the perfect bacterial genome using Oxford Nanopore and Illumina sequencing. https://preprints.scielo.org/index.php/scielo/preprint/view/5053/9840

# Sample types

Sample types covered are Gram negative and Gram positive cells, white blood cells from whole blood, cultured mammalian cells and whole blood (Figure 1). Because the column extract gets depleted during the extraction process no size selection is necessary prior to ONT library preps. Fire Monkey HMW-DNA is library ready and can enable a complete assembly with ~1000x coverage of the chromosome and recovery of a ~130kb plasmid one 1 MinION flow cell (Bacteria, Figure 2), as well as generate 160Gb of total data with 40x coverage at an N50 of 51kb on a PromethION flow cell (Mammalian, Figure 3).

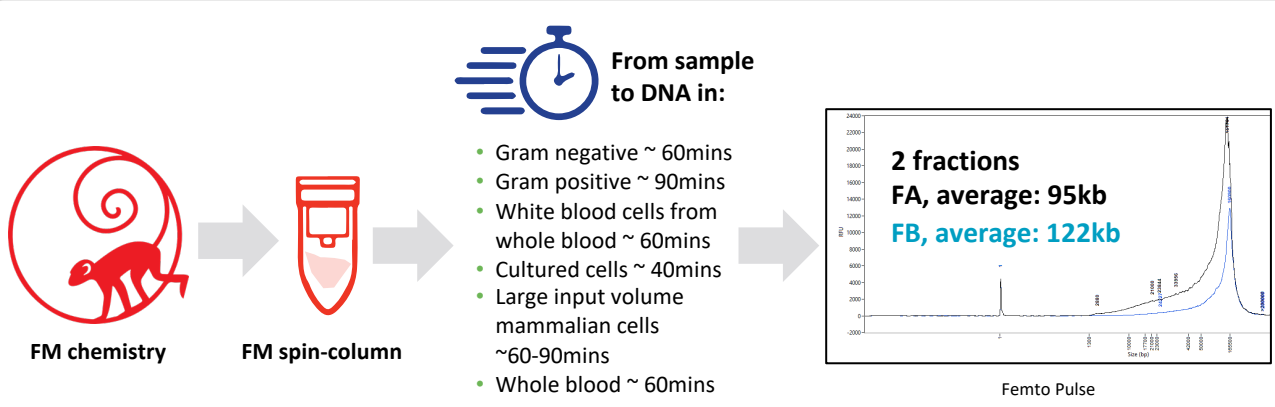



**Figure 1.** Sample types covered by the Fire Monkey HMW-DNA spin-column protocols. Fire Monkey extracts HMW-DNA with average strand lengths of appx. 100kb and above for Fraction A (FA) and Fraction B (FB) from bacterial and mammalian cell samples. The extract does not require size selection prior to ONT library preps.

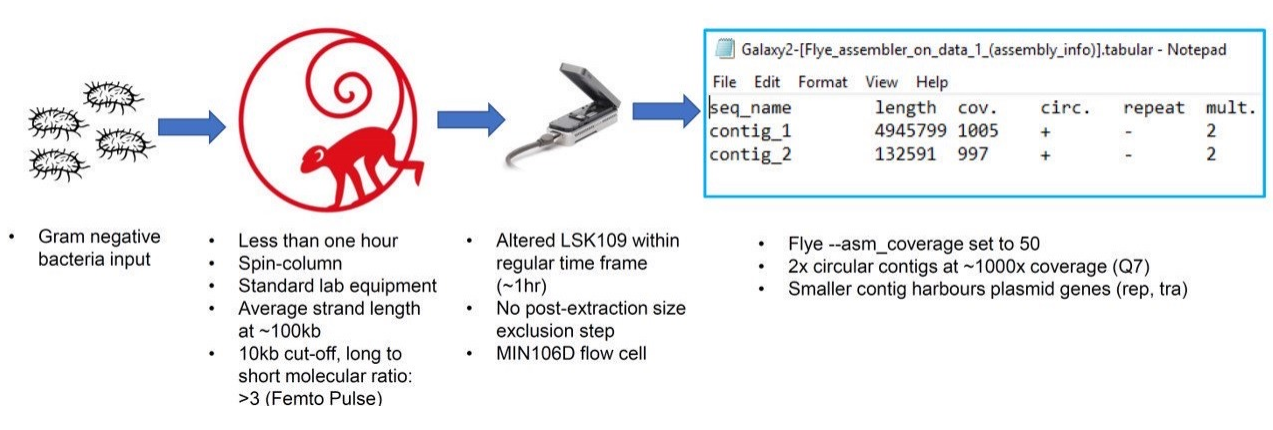



**Figure 2.** An E *coli* Fire Monkey extract (Fraction B) ran on LSK109/MinION enabled a complete assembly with ~1000x coverage of the chromosome (Flye, Q7) and recovery of a ~130kb plasmid. No size selection was performed post-extraction.

**Run statistics**

| 96hrs run | Throughput (Gb) | Reads # | Read N50 (kb) |
|---|---|---|---|
| All data | 160.3 | 12,952,997 | 38,6 |
| Pass data Q>7 | 136.5 | 10,262,206 | 40,3 |
| 100kb+ data Q>7 | 13.3 | 108,878 | 118,8 |
| **Top 40x coverage** | **109.42** | **2,907,649** | **51,5** |

- Sample type : horse white blood cells from 1ml blood
- 1 hour spin column Fire Monkey
- No size selection post extraction (pooled Fractions + B)
- LSK110 / Single PromethION flow cell
- 96hrs run, 3 flushes
- 160.3Gb of total data, 15Gb within last 24hrs
- 13.3Gb 100kb+ Q>7
- Top 40x coverage with read N50: 51.5kb
- Horse genome is ~2.5 billion bases long
- Genome assembled in 759 contigs with a contig N50 of ~25.5 million bases long (longest contig ~86.8 millions bases)

**Filtered Canu assembly**

| Horse genome length (Gb) | Number of contigs | Contig N50 (kb) | Longest contig (kb) |
|---|---|---|---|
| 2.562 | 759 | 25,481 | 86,755 |

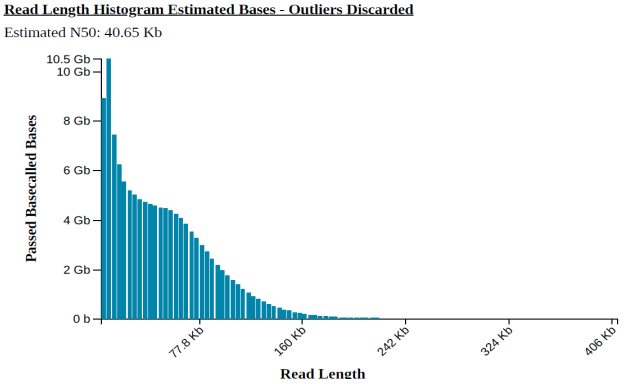
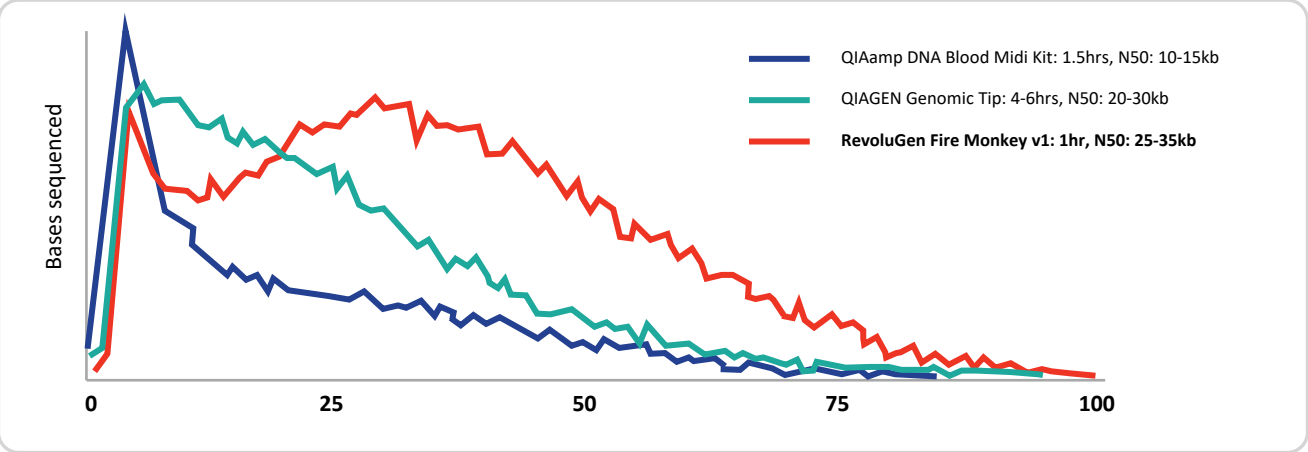



**Figure 3.** A white blood cell Fire Monkey extract ran on LSK110/PromethION enabled 40x coverage at N50: 51kb. No size selection was performed post-extraction.

# External validation

Both mammalian and bacterial protocols have been validated on ONT, Illumina and 10x Genomics workflows and results have been published in peer-reviewed journals. Validation of the original version of the kit was performed by ONT in 2018 and is continuously being carried out by several KOLs including UKHSA and Quadram Institute labs etc. The potential for multiplexing bacterial isolate samples has been recently demonstrated with 48x-plex Salmonella MinION runs dropping sequencing cost to appx $20 per genome (Figure 4).

## Fire Monkey v1 – Oxford Nanopore first validation in Feb 2018




- Oxford Nanopore Technologies validated the original version of the kit on white blood cells against two Qiagen products
- Fire Monkey's first generation product demonstrated the highest read length quality (N50) in a fraction of the time used by Qiagen's industry standard product
- Current kit version generates N50 values of 40-50kb+ for the same sample type

## Latest Fire Monkey version - Quadram Institute - April 2022

MICROBIOLOGY SOCIETY

ANNUAL CONFERENCE 2022

04 - 07 April 2022

- Quadram scientists presented their work at the Microbiology Society Annual Conference in Belfast
- Fire Monkey spin column was successfully used **to multiplex 48 Salmonella isolates on a single MinION flow cell, dropping the sequencing cost to appx. $20 per genome[1]**
- Complete genome assemblies with 30x theoretical coverage

**Figure 4.** External validation.

*[1]Library and MinION flow cell price considered to be appx. $1000*

# External validation

| Institute / Organization | Sequencing Platform | Publication |
|---|---|---|
| Quadram Institute | illumina | Emergence of Resistance to Fluoroquinolones and Third-Generation Cephalosporins in Salmonella Typhi in Lahore, Pakistan<br>Rasheed F et al, (2020) Microorganisms 8 (9) 1336 |
| UEA Norwich Medical School; Quadram Institute | Oxford Nanopore Technologies | Emergence of ciprofloxacin heteroresistance in foodborne Salmonella enterica serovar Agona.<br>Zhang CZ et al, (2020) Journal of Antimicrobial Chemotherapy 75(10) 2773 |
| Canadian Food Inspection Agency; UK Health Security Agency | Oxford Nanopore Technologies | Complete Genome Assemblies of the Rare Salmonella enterica Serovar Adjame Using Nanopore and Illumina Sequence Reads<br>Gao R et al (2020),Microbiol Resour Announc 9(35): e00280-20 |
| SpeeDx | PCR | Nucleic Acid Ratio Determination (Patent Application)<br>Todd AV & Lima NE (2020) Pub. No.: US 2020/0199651 |
| Dana-Farber Cancer Institute; Harvard University | 10X Genomics; illumina | Determination of complete chromosomal haplotypes by bulk DNA sequencing<br>Tourdot RW et al, (2021) Genome Biology. Volume 22, Article 139 |
| University of Bath; Roslin; The University of Edinburgh; UK Health Security Agency | Oxford Nanopore Technologies | Acquisition and loss of CTX-M plasmids in Shigella species associated with MSM transmission in the UK<br>Locke R et al, (2021) Microbial Genomics. Volume 7, Issue 8 |
| UK Health Security Agency | Oxford Nanopore Technologies | Analysis of a small outbreak of Shiga toxin-producing Escherichia coli O157:H7 using long-read sequencing<br>Greig DR et al, (2021) Microbial Genomics. Volume 7, Issue 3 |
| UEA Norwich Medical School; Quadram Institute; UK Health Security Agency; Roslin | Oxford Nanopore Technologies | Characterization of a pESI-like plasmid and analysis of multidrug-resistant Salmonella enterica Infantis isolates in England and Wales<br>Lee WWY et al, (2021) Microbial Genomics. Volume 7, Issue 10 |
| Liverpool School of Tropical Medicine | Oxford Nanopore Technologies | First identification of bla NDM-5 producing Escherichia coli from neonates and a HIV infected adult in Tanzania<br>Manyahi J et al, (2022) Journal of Medical Microbiology. Volume 71, Issue 2 |
| UK Health Security Agency | Oxford Nanopore Technologies | Use of Nanopore Sequencing to Characterise the Genomic Architecture of Mobile Genetic Elements Encoding blaCTX-M-15 in Escherichia coli Causing Travellers' Diarrhoea<br>Bird MT et al, (2022) Front. Microbiol. Volume 13, Article 862234 |
| Quadram Institute | Oxford Nanopore Technologies | Impact of Salmonella genome rearrangement on gene expression<br>Waters EV et al, (2022) Evolution Letters Volume 6, Issue 6, pages: 426-437 |
| UK Health Security Agency; Quadram Institute | Oxford Nanopore Technologies | Dynamics of Salmonella enterica and antimicrobial resistance in the Brazilian poultry industry and global impacts on public health<br>Alikhan NF et al, (2022) PLOS Genetics 18(6): e1010174 |

# External validation

| Institute / Organization | Sequencing Platform | Publication |
|---|---|---|
| UK Health Security Agency | Oxford Nanopore Technologies | Outbreak of sexually transmitted, extensively drug-resistant Shigella sonnei in the UK, 2021-22: a descriptive epidemiological study Charles H et al, (2022) The Lancet Infectious Diseases 22: 1503-1510 |
| UK Health Security Agency; Quadram Institute | Oxford Nanopore Technologies | Characterization of a P1-bacteriophage-like plasmid (phage-plasmid) harbouring bla CTX-M-15 in Salmonella enterica serovar Typhi Greig DR et al, (2022) Microbial Genomics Volume 8, Issue 12 |
| Liverpool School of Tropical Medicine | Oxford Nanopore Technologies; illumina | Intracellular Transposition of Mobile Genetic Elements Associated with the Colistin Resistance Gene mcr-1 Goodman RN et al, (2022) Microbiology Spectrum e03278-22 |
| USGS science for a changing world | 10x Genomics; illumina | Multi-omics responses in tree swallow (Tachycineta bicolor) nestlings from the Maumee Area of Concern, Maumee River, Ohio Tseng CY et al, (2023) Science of The Total Environment Volume 856, Part 2, 159130 |

# Automation

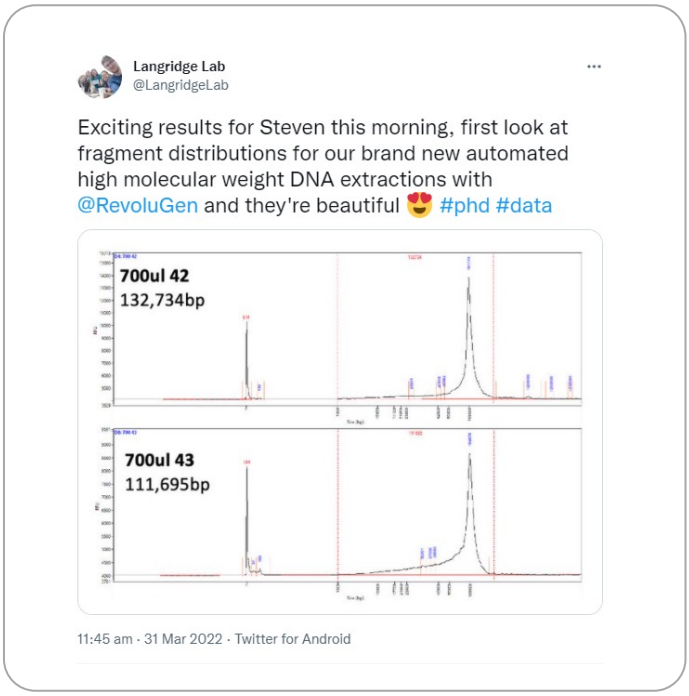
The automated Fire Monkey version substitutes spin columns with 96 well plates and spinning with positive pressure on a benchtop Resolvex A200 instrument (Tecan). The workflow automates loading, washing and eluting HMW-DNA from the filter wells with an average of 100kb and above for both mammalian and bacterial samples. An automated Fire Monkey system has been transferred to Quadram, a leading UK research Institute, to enable automation of HMW-DNA extraction across high volume bacterial sequencing projects in pathogen persistence and antimicrobial resistance (AMR) monitoring (Figure 5).


## Automated Fire Monkey workflow

- Bespoke Fire Monkey 96-well plates replace single columns & positive pressure adaptation replaces centrifugation and automates all loading, washing and elution steps
- Benchtop Tecan Resolvex A200 positive pressure instrument
- HMW-DNA with average strand length of 100kb+

## External validation

- A Quadram Intistute group is an early adapter of RevoluGen's automated solutions
- Urinary Track Infection *E coli* samples generated average strand lengths of 100kb and above




*With the automation by RevoluGen we will be able to prep tens of bacterial isolates at a time. This increases our research capacity and capability quite dramatically.*

*We look forward to further expanding our relationship with RevoluGen to explore the role of its technology in metagenomic analysis and to explore the wider benefits of automation.*

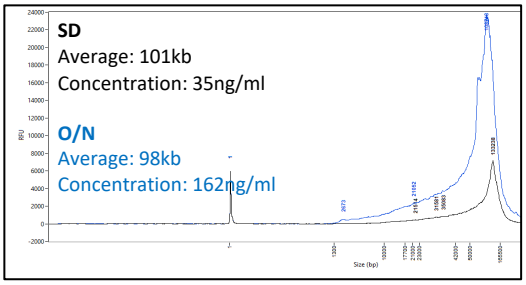
Quadram Institute Science Health Food Innovation

**Figure 5.** Fire Monkey 96-well plates and workflows have been adapted to extract HMW-DNA using the benchtop positive pressure Resolvex A200 from Tecan.
The automated RevoluGen system has been validated at the Quadram Institute.

# New protocols

Depending on sample type and sample volume processed overnight (O/N) treatment with the elution buffer may be required to release all HMW-DNA from the column matrix. In some cases appx 4x more HMW-DNA can be extracted with O/N treatment (*E coli*, Figure 6). Fire Monkey protocols have also been developed to extract HMW-DNA from large sample volumes, such as white blood cells from up to 5ml of blood (Figure 6). In both cases the user can extract more HMW-DNA which can be stored for several sequencing runs on different sequencing platforms. These options help save on storage space and extraction cost.

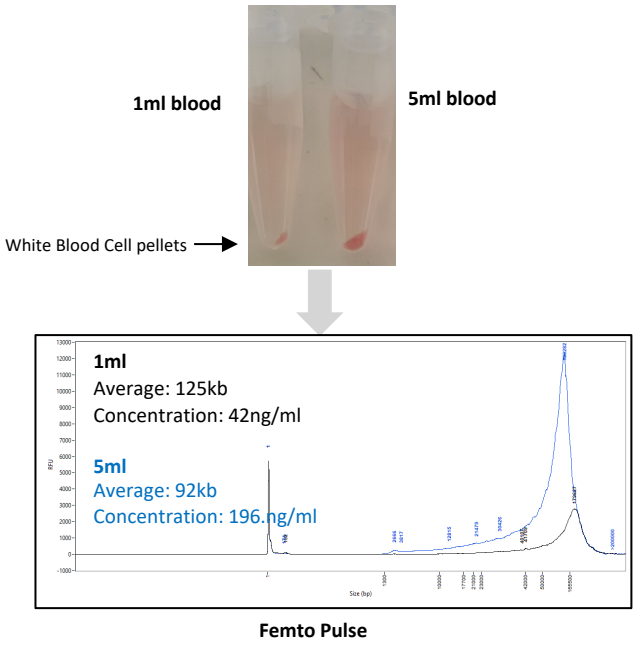
## Overnight elution




**Femto Pulse**

Overnight (O/N) elution boost HMW-DNA mass extracted in comparison to Same Day (SD) elution for 1ml *E coli* culture. Pooled Fractions A + B.

## Large input volume




**Femto Pulse**

HMW-DNA from white blood cell pellets from 1 and 5ml of horse blood. Pooled Fractions A + B.

**Figure 6.** Overnight elution and large input volume protocols extract more-HMW-DNA.

# Protocols in development

Sequencing throughput can be enhanced for Nanopore by reducing average input length and PacBio HiFi workflows require a tailored tight distribution. In both cases users will extract HMW-DNA which will then be fragmented (e.g. Megaruptor instrument) and size depleted. This is time consuming, costly and also wastes a lot of DNA material. Tuneable Fire Monkey protocols extract, fragment and size deplete DNA in a single ~1hr workflow. Cells are lysed and the lysate is fragmented through needle re-suspension prior to column purification. This can result in 40Gb on a single MinION flow cell/run and 19.3Gb of HiFI reads for mammalian genomes (Figure 7).

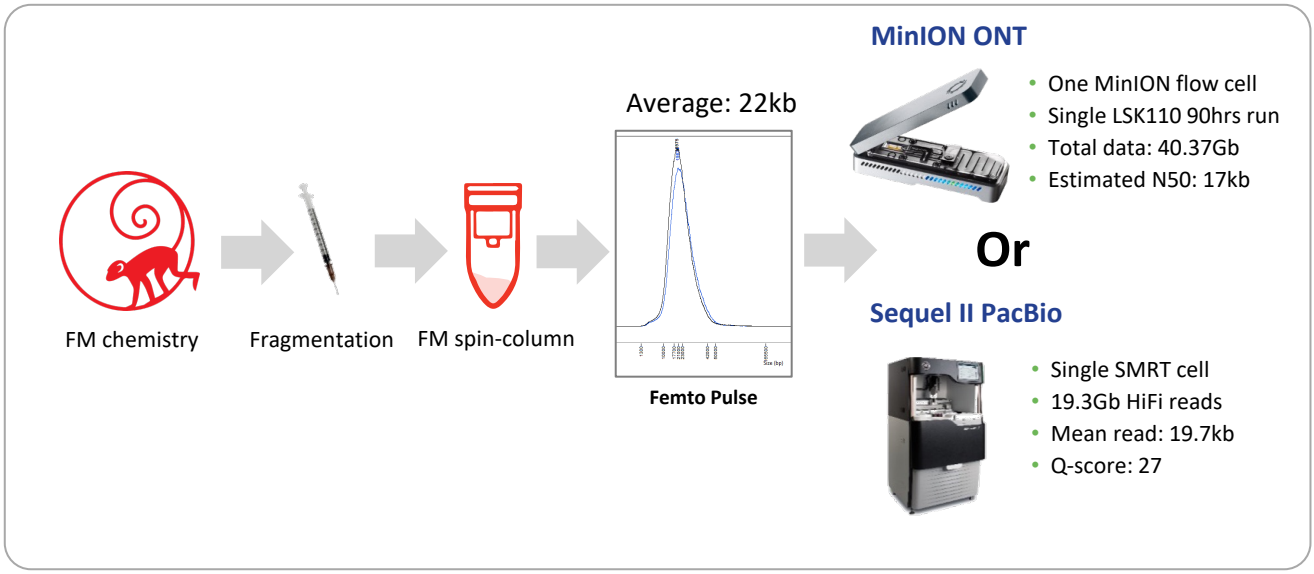



**Figure 7.** Parallel extraction/fragmentation protocol in development. Single workflow extracts tailored DNA for ONT Ligation and PacBio HiFi protocols. Contact : info@revolugen.co.uk for protocol information.

## Simpler operation

High Molecular Weight DNA from an easy, familiar spin column format

## Faster processing

Immediately ready to use in one hour and that includes size selection

## Better results

Structural clarity from the average DNA lengths of >100kb

## Cheaper sequencing

Big yield of Not too short/Not too long DNA cuts $/bacterial genome costs

HOW TO ORDER


## Fire Monkey 10 Column Kit

To place an order and for more information please Click here