

REVOLUGEN

Fire Monkey/Fire Flower is a spin-column HMW extraction & size selection kit developed by RevoluGen.

- **Fire Monkey** takes ~1hr and produces N50s of over 50Kb for mammalian cells, and up to 20Gb for bacteria. Complete chromosome assembly and full plasmid recovery can be achieved with 50x coverage using fragments >122kb, and 1000x coverage after one polishing step.
- **Fire Flower** is a ~15mins spin column process that can be used for size-selection of already extracted DNA and can more than double N50 values and reads above 100kb.

From sample to DNA in **1h**

Spin column Standard format

> 100kb Average extract size

FIRE MONKEY HMW-DNA extraction kit

Bacteria cells throughput up to **20 Gb**

10-20kb Size selection Cut-off

N50 mammalian cells up to **62 kb** **XY**



Standard equipment

Quick protocol **15m**

Spin column Standard format

Removes DNA fragments < 10kb

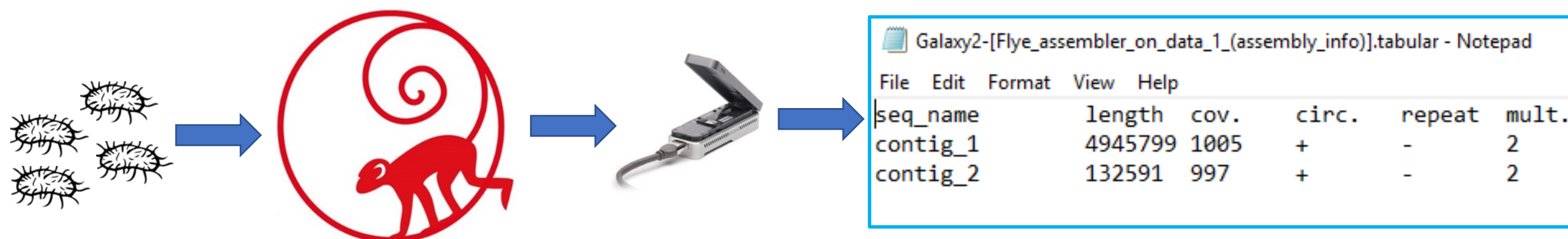
FIRE FLOWER DNA size selection kit

5x Bigger MW cut-off vs SPRI beads

COMPATIBLE with all existing NAIP kits

DOUBLE N50 and average read

An **E.Coli Fire Monkey** extract ran on LSK109 enabled a complete assembly with ~1000x coverage of the chromosome (Flye, Q7) and recovery of a ~130kb plasmid (Q7, ~1000x coverage) using LSK109.

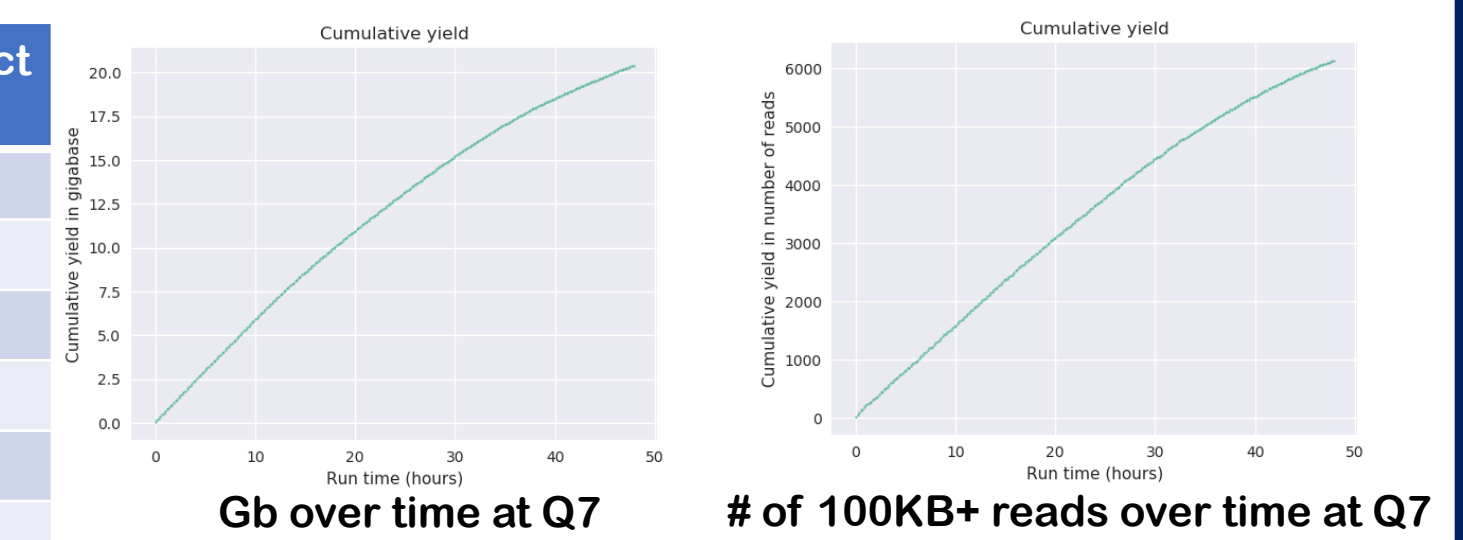


- Gram negative bacteria input
- Less than one hour
- Spin-column
- Standard lab equipment
- Average strand length at ~100kb
- 10kb cut-off, long to short molecular ratio: >3 (Femto Pulse)
- Altered LSK109 within regular time frame (~1hr)
- No post-extraction size exclusion step
- MIN106D flow cell
- Flye --asm_coverage set to 50
- 50x coverage with fragments >122kb
- 2x circular contigs at ~1000x coverage (Q7), after one polishing step
- Smaller contig harbours plasmid genes (rep, tra)

Informatics performed by Dr Gemma Langridge, Quadram Institute Bioscience, United Kingdom.

Escherichia Coli cells pre-treated in 100µl of 1x DNA/RNA Shield (Zymo Research) used to extract DNA. **Fire Monkey** Fraction B generates 20Gb+ and >6,000 100KB+ reads at Q7 on LSK109/MinION. Long reads are less reliant on the cold chain.

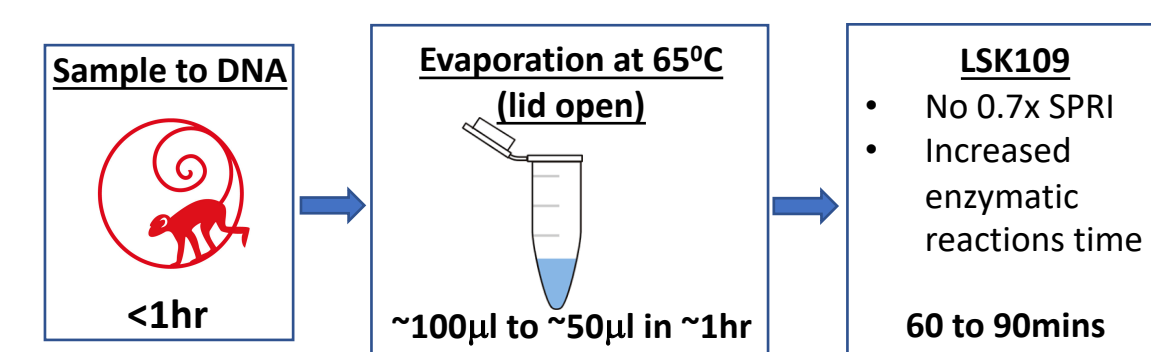
600 million <i>Escherichia coli</i> (pre-treated in DNA/RNA Shield) DNA extract sequenced on R9.4.1/LSK109/MinION for 48hrs - NanoPlot analysis		
Q-score cut-off	≥7	≥11
N50 (bp)	40,617	41,116
Mean read length (bp)	20,943	23,653
Mean read quality	10.1	11.4
# of reads	973,397	314,684
Total bases	20,386,140,824	7,443,343,404
Top read (bp)/[Q-score]	301,791/[7.6]	230,282/[11.4]
# of reads ≥50,000bp/[Yield in Gb]	106,908/[7.3]	39,681/[2.7]
# of reads ≥100,000bp/[Yield in Gb]	6,130/[0.7]	1,970/[0.2]



- Pellet treated for 5mins at RT with 100µl of DNA/RNA Shield
- <1hr sample to DNA (20mins Proteinase K lysis, ~15mins spins)
- Fraction B at 100µl elution volume
- No post-extraction size selection
- 48µl of extract used
- No nuclease flush
- 48hrs run

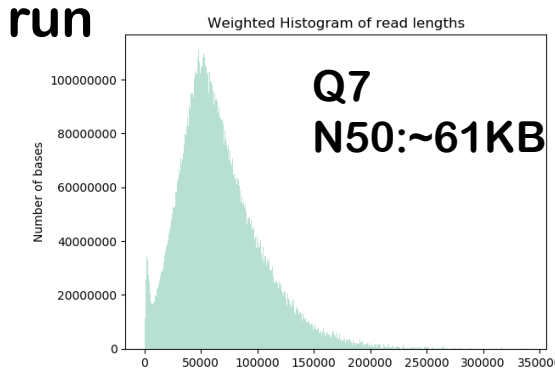
2x Fire Monkey horse genome (2.7Gb) fraction B replicates, concentrated by evaporation, generate 15.5Gb, N50~61KB and >20,000 100KB+ reads on LSK109/MinION, no post-extraction size selection. Single nuclease flush.

Fraction	Average length pre-evaporation	Average length post-evaporation	Mass loaded to flow cell (Nanodrop)
Pooled A1+A2	85,087bp	-	-
B1	112,260bp	113,941bp	~500ng
B2	107,147bp	99,700bp	~500ng



1ml horse blood DNA extracts sequenced on R9.4.1/LSK109/MinION for 48hrs - NanoPlot analysis		
Q-score cut-off	≥7	≥11
N50 (bp)	60,855	62,363
Mean read length (bp)	36,050	42,029
Mean read quality	11.1	11.8
# of reads	429,986	223,560
Total bases	15,501,185,846	9,396,111,073
Top read (bp)/[Q-score]	339,062/[12.1]	339,062/[12.1]
# of reads ≥50,000bp/[Yield in Gb]	129,001/[10]	81,412/[6.3]
# of reads ≥100,000bp/[Yield in Gb]	20,879/[2.6]	13,093/[1.6]

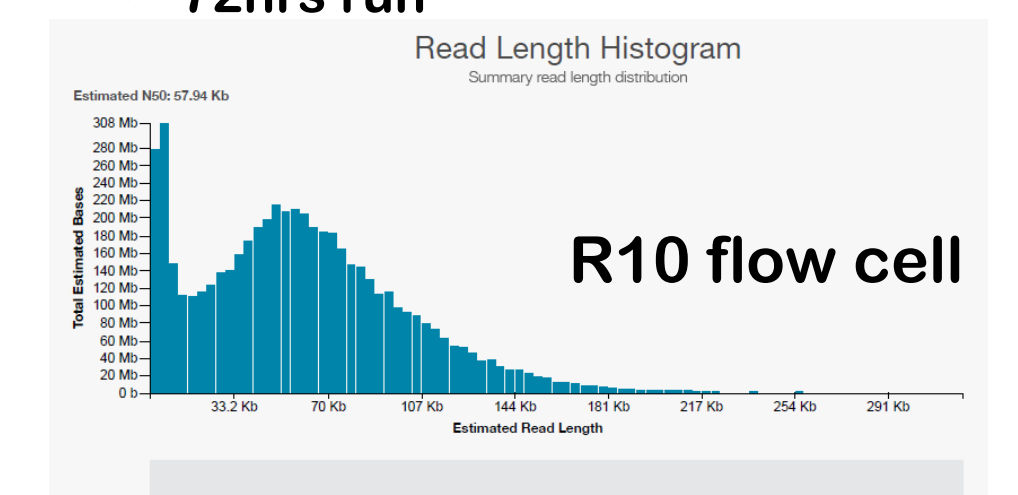
- <1hr sample to DNA (~20mins RBC lysis, 10mins Proteinase K lysis, ~15mins spins)
- Fraction B at 80µl elution volume; concentrated by evaporation
- No post-extraction size selection
- Altered LSK109
- Single nuclease flush
- 48hrs run



Fire Monkey horse genome (2.7Gb) fraction A generates N50~62KB and >6000 100KB+ reads on R10/LSK109/MinION, no post-extraction size selection. Yield at 4.4Gb but nuclease flushes can push it higher.

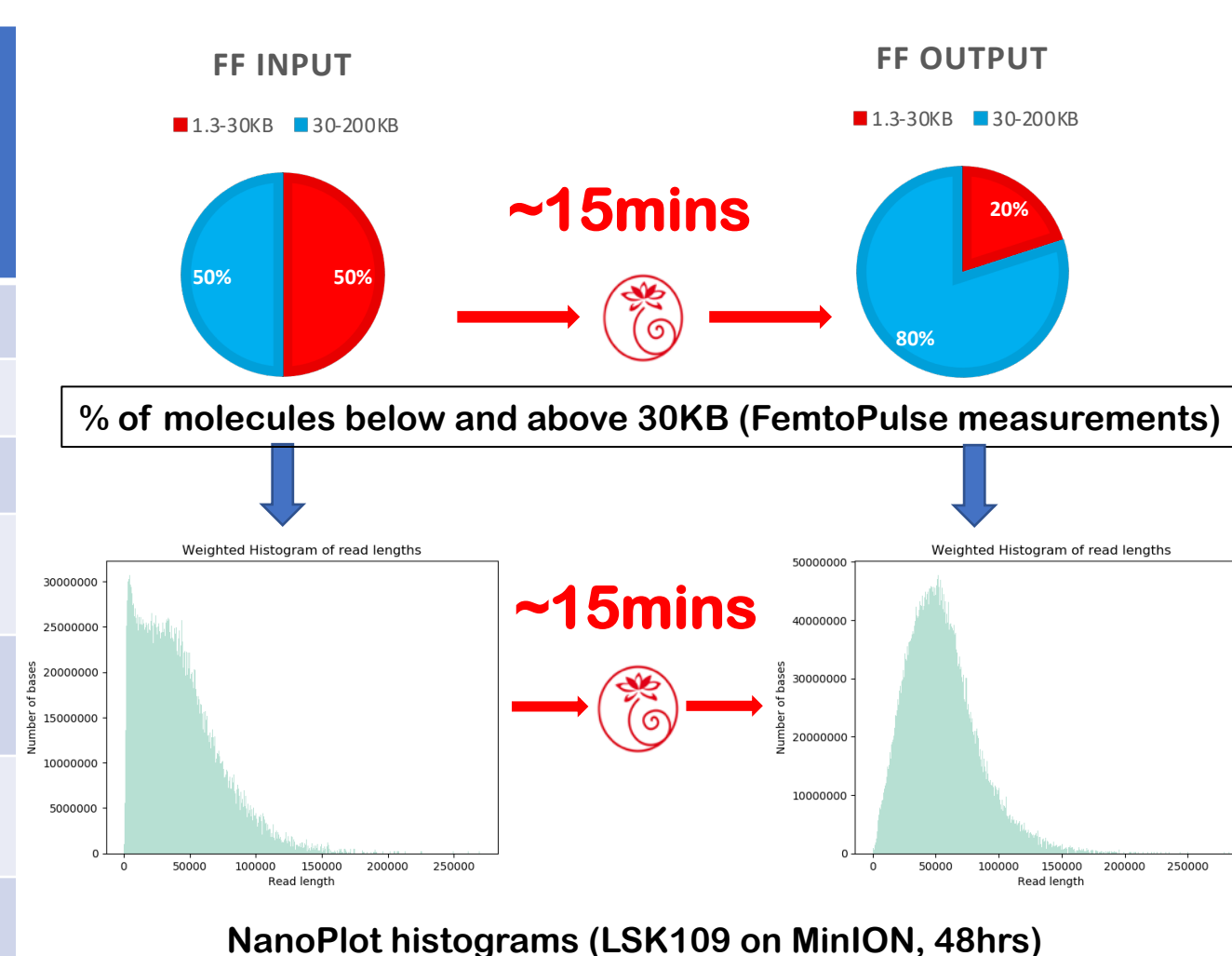
White blood cells (2ml horse blood) DNA extract sequenced on R10/LSK109/MinION - NanoPlot analysis	
Q-score cut-off	≥7
N50 (bp)	61,753
Mean read length (bp)	24,966
Mean read quality	7.9
# of reads	176,204
Total bases	4,399,157,969
Top read (bp)/Q-score	362,394/8.4
# of reads ≥50,000bp/Throughput (Gb)	34,814/2.77
# of reads ≥100,000bp/Throughput (Gb)	6,474/0.8

- <1hr sample to DNA (~20mins RBC lysis, 10mins Proteinase K lysis, ~15mins spins)
- Fraction A at 100µl elution volume
- No post-extraction size selection
- Altered LSK109
- No nuclease flush
- 72hrs run



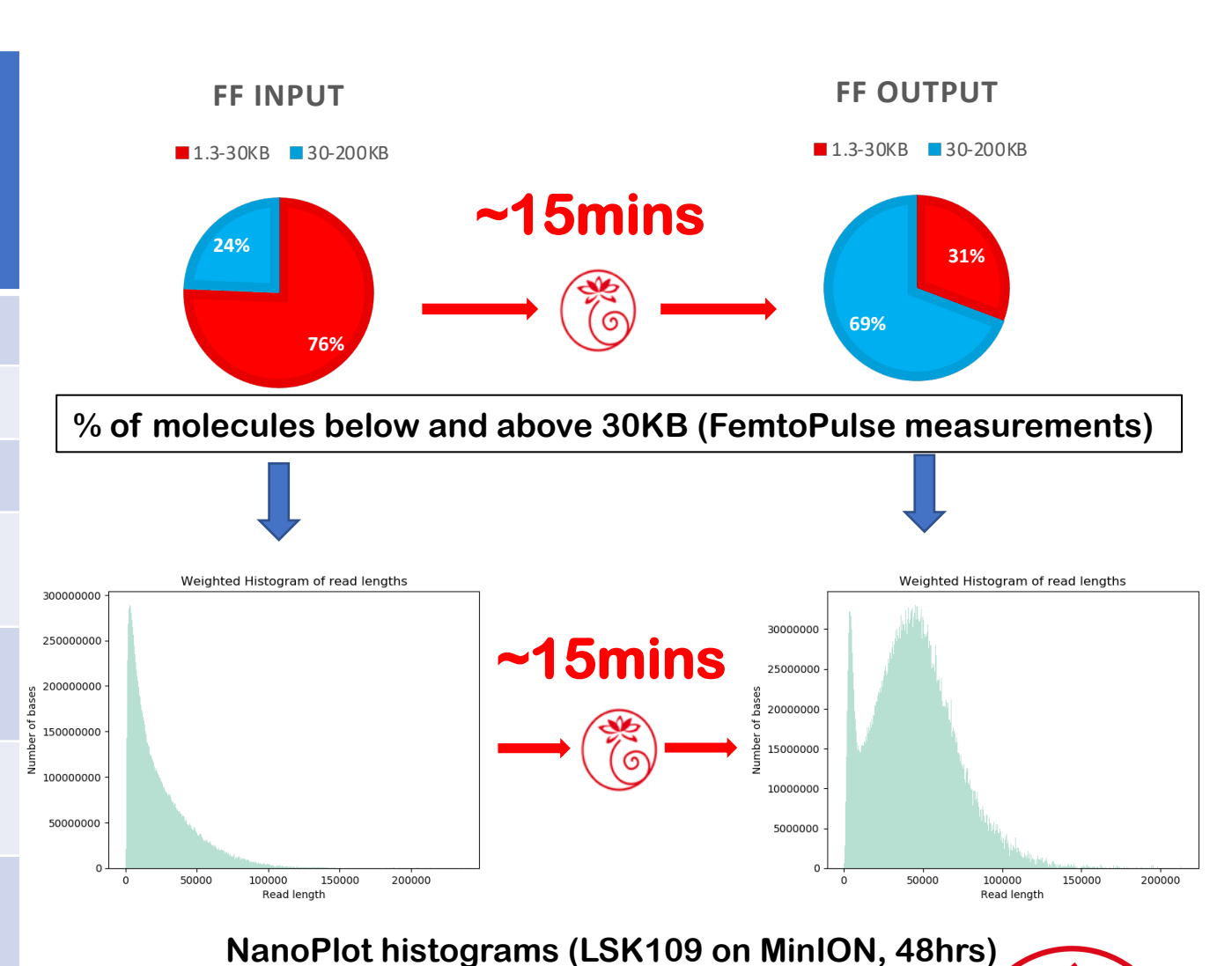
Fire Flower size selects on up to 200µl of extracted DNA. Fire Flower increases the % of molecules above 30kb and ~doubles most long read metrics. (Results from 48hrs runs on LSK109/MinION).

NanoPlot (LSK109 on MinION, 48hrs)	FF Input	FF output	Long fragment metrics X increase
Average (bp)	17,012	37,163	2.18
Q-score	10.6	10.7	-
N50 (bp)	36,919	51,959	1.4
Total # of reads	215,077	161,295	-
Total yield (Gb)	3,69	5,99	-
# reads >50kb	17,460	44,917	2.57
# reads >100kb	1,575	3,713	2.35
# reads >150kb	123	284	2.3
Top read	269,583	282,982	-



15mins of Fire Flower treatment on a very challenging DNA input sample more than doubles average read length and N50 values. (Results from 48hrs runs on LSK109/MinION).

NanoPlot (LSK109 on MinION, 48hrs)	FF Input	FF output	Long fragment metrics X increase
Average (bp)	7,762	20,963	2.7
Q-score	10.1	10	-
N50 (bp)	16,901	43,521	2.57
Total # of reads	1,738,300	191,735	-
Total yield (Gb)	13,49	4	-
# reads >50kb	26,322	23,850	0.9
# reads >100kb	1,073	1,110	1.03
# reads >150kb	29	45	1.55
Top read	236,025	213,123	-



- **Fire Monkey** is an extremely rapid HMW DNA extraction kit. It extracts average DNA fragment lengths of between 100-130kb (Femto Pulse), with significantly reduced numbers of small DNA fragments shorter than 10kb. This results in high throughput and high N50 values.
- **Fire Flower** size-selects already extracted DNA (process time ~ 15 mins) It can more than double N50 values and reads above 100kb.

Contact: georgios@revolugen.co.uk for a free kit, quoting NCM 2019 New York.



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