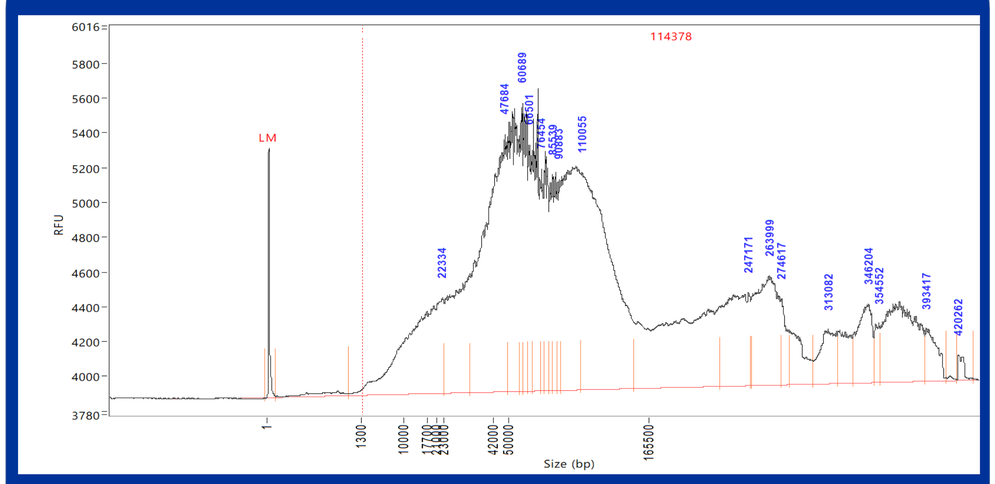
REVOLUGEN

FIRE MONKEY
High Molecular Weight DNA Extraction

FIRE FLOWER
Size Selection

Fire Monkey extracts High Molecular Weight (HMW) DNA from bacterial and mammalian cells within 1hr

High Molecular Weight DNA extraction above 100kb



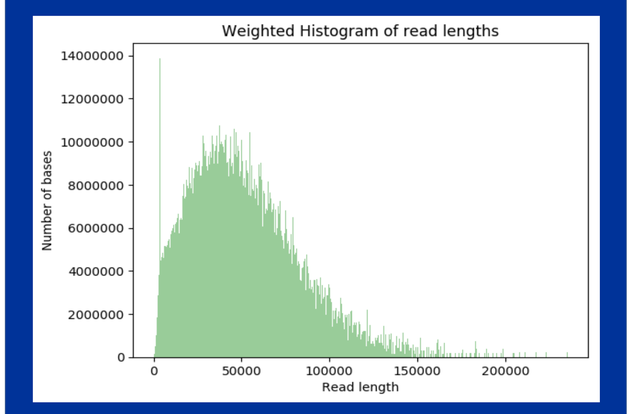
Agilent Femto Pulse trace (extended run) of a white blood cell Fire Monkey extract (average: 114,378bp).

Fire Monkey Extraction generates N50 values of ~50kb

Fire Monkey white blood cell DNA extract sequenced on LSK109 (ONT)				
Sequencing time	6hrs		48hrs	
Q-score cut-off	≥7	≥11	≥7	≥11
N50 (bp)	**47,606**	**50,553**	**49,590**	**52,316**
Mean read length (bp)	27,080	29,748	30,096	32,107
Mean read quality	10.2	11.3	10.1	11.3
Number of reads	54,561	13,965	156,879	37,115
Yield (Gb)	1.47	0.41	4.72	1.19
Top read (bp)	235,546	211,212	308,648	211,212

47μl (instead of the ONT recommended 20μl) of a Fire Monkey white blood cell extract DNA extract were sequenced according to the ONT LSK109 protocol without a 0.7x SPRI post-extraction step (MinION, FLO-MIN106D R9 Version).

NanoPlot Read Lengths



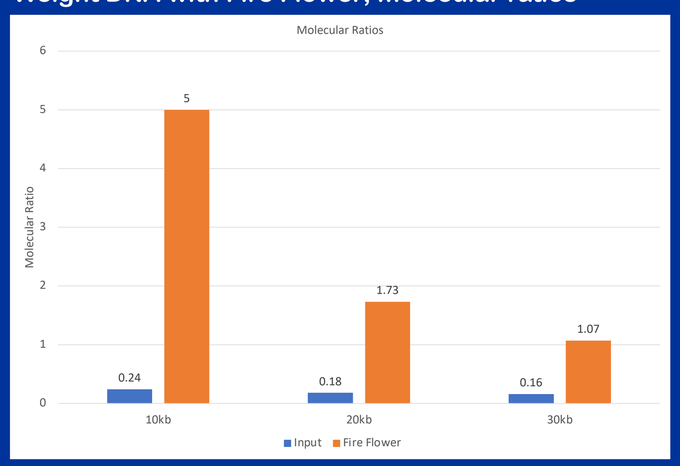
NanoPlot generated histogram of number of bases vs read length for 48hrs of sequencing with a ≥7 Q-score cut off.

- Only basic lab equipment required.

- Fire Monkey is a simple spin-column process which extracts HMW-DNA with average strand lengths of **100kb and above** for both bacteria and mammalian cells **within 1hr**.
- Protocols are user-friendly and can be high-throughput.
- Due to Fire Monkey's **in-built size-exclusion aspect** very few fragments below 10kb will be extracted. As a result post-extraction size-exclusion steps that are time-consuming and could break long DNA fragments are not necessary, meaning user-friendly sequencing protocols and better sequencing quality results.
  - For example, **N50 values of ~50kb can be achieved** without the ONT recommended 0.7x SPRI step for LSK109 sequencing.

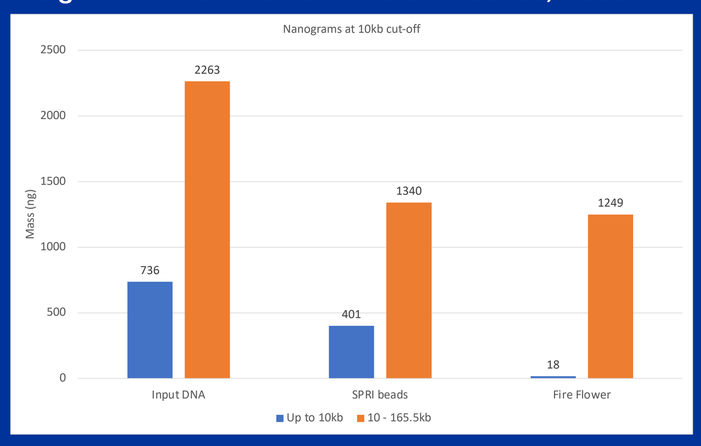
Fire Flower size selects extracted DNA from all sample types within 15mins

Increase in High Molecular Weight vs Low Molecular Weight DNA with Fire Flower; molecular ratios



An input sample consisting of Low Molecular Weight DNA mixed with High Molecular Weight DNA (total mass: ~3μg) was used as the starting point for the Fire Flower size-selection process. The DNA output (final mass: ~1μg) had an increase in average strand length. Input average DNA length was 70kb vs the Fire Flower output of 106kb (Agilent Femto Pulse measurements). The Agilent Femto Pulse software was then used to calculate the molecular ratio of the number of molecules with lengths longer than and shorter than the 10kb, 20kb and 30kb measurement thresholds. The Molecular ratio is defined as the number of long molecules above the threshold point divided by the number of molecules below the size threshold point.

Increase in High Molecular Weight vs Low Molecular Weight DNA with Fire Flower vs SPRI beads; mass



An input sample consisting of Low Molecular Weight DNA mixed with High Molecular Weight DNA (total mass: 3μg) was used as the starting point for the Fire Flower size-selection process vs 0.7x SPRI beads. After size exclusion Nanodrop and Agilent Femto Pulse analysis of the DNA output were used to calculate the number of nanograms below and above 10kb. Both SPRI and Fire Flower reduce the mass of over 10kb at a similar rate. However, Fire Flower has a much greater efficiency in reducing the below 10kb mass than SPRI.

- Only basic lab equipment required.

- Fire Flower is a simple spin-column process which size selects extracted DNA from all sample types **within 15mins**. Protocols are user-friendly and can be high-throughput.
- Fire Flower can increase the overall average strand length **by 30%** (assessed by Agilent Femto Pulse), while depleting DNA fragments **up to 30kb**.

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