Fully automated bead-based High Molecular Weight DNA extraction for long-read sequencing

Maryia Karpiyevich[1], Georgios Patsos[2], Graeme Smethurst[1], Kamila Koprowska[1]

[1]SPT Labtech, Melbourn, Cambridgeshire, UK
[2]RevoluGen, Hadfield, Derbyshire, UK

introduction

The growing popularity of long read sequencing (LRS) is driving up demand for High Molecular Weight (HMW) DNA. However, the extraction of HMW DNA has proven to be a barrier for routine uses. Many conventional methods are expensive, resource- and time-intensive, and do not produce DNA of sufficient quantity and quality for LRS. Moreover, they might be challenging to scale up due to not being automation-friendly or requiring specialized equipment and labware, like vacuum manifolds or wide bore tips, driving the costs up.

Here, we describe a novel automated plate-based method of extracting HMW DNA that removes these obstacles. Combining RevoluGen® bead-based Fire Monkey® chemistry with the all-in-one SPT Labtech firefly® liquid handler allows researchers to rapidly extract high-quality HMW DNA with minimal hands-on time. The single-step method utilizes silica-coated magnetic beads and patent-protected chemistry to capture the long DNA fragments with a significantly reduced number of small fragments, so that the extract does not require laborious and wasteful size selection processes post-extraction. The DNA extracted on firefly can be used directly for library preparation and subsequent sequencing. Here we demonstrate this on Oxford Nanopore Technologies (ONT) systems.

highlights

- This method delivers high-quality HMW DNA for Nanopore library preparation and sequencing in under 2 hours.
- This simplified protocol does not require post-extraction sample shearing or size selection.
- By combining technologies for pipetting, non-contact dispensing and shaking within a compact benchtop footprint, firefly replaces the need for multiple instruments for this extraction workflow and preserves precious laboratory space.
- The method yields DNA highly enriched for molecules of 100kb and larger, in quantities sufficient for ONT sequencing.
- Automation with firefly allows processing from 1 to 96 or more samples in parallel, enabling large-scale bacterial surveillance and antibiotic microbial resistance studies.
- Replacing pipette-driven reagent additions with firefly's non-contact dispensing reduces the amount of single-use plastic consumables, making the whole protocol not only faster but also more environmentally friendly.

REVOLUGEN | sptlabtech

method

200 µL aliquots of overnight *E.coli* NCTC 13400 culture were used to extract HMW DNA on firefly. Frozen *E.coli* pellets were defrosted, resuspended in STET buffer supplemented with lysozyme and loaded into a 96-well plate. The subsequent magnetic bead-based extraction of HMW DNA utilized RevoluGen Fire Monkey chemistry and was fully automated on the firefly instrument (Figure 1 and Figure 2).

Control (CTRL) samples were prepared from the same lot of *E.coli* following the equivalent manual protocol with one modification. After washing, the samples went through two rounds of elution, in 20 µL of Elution Buffer (EB) each vs 2x 14 µL in the automated protocol. In total, 9 pellets were processed by three lab members in triplicate. The CTRL samples were pooled before further analysis and sequencing.

Fragment size distribution and HMW DNA yields were determined using the Femto Pulse System (Agilent Technologies). Libraries were prepared from 8 pooled firefly samples and pooled CTRL samples using Ligation Sequencing Kit V14 (SQK-LSK114) and subsequently sequenced on ONT MinION instrument with a R10 flow cell (FLO-MIN114).

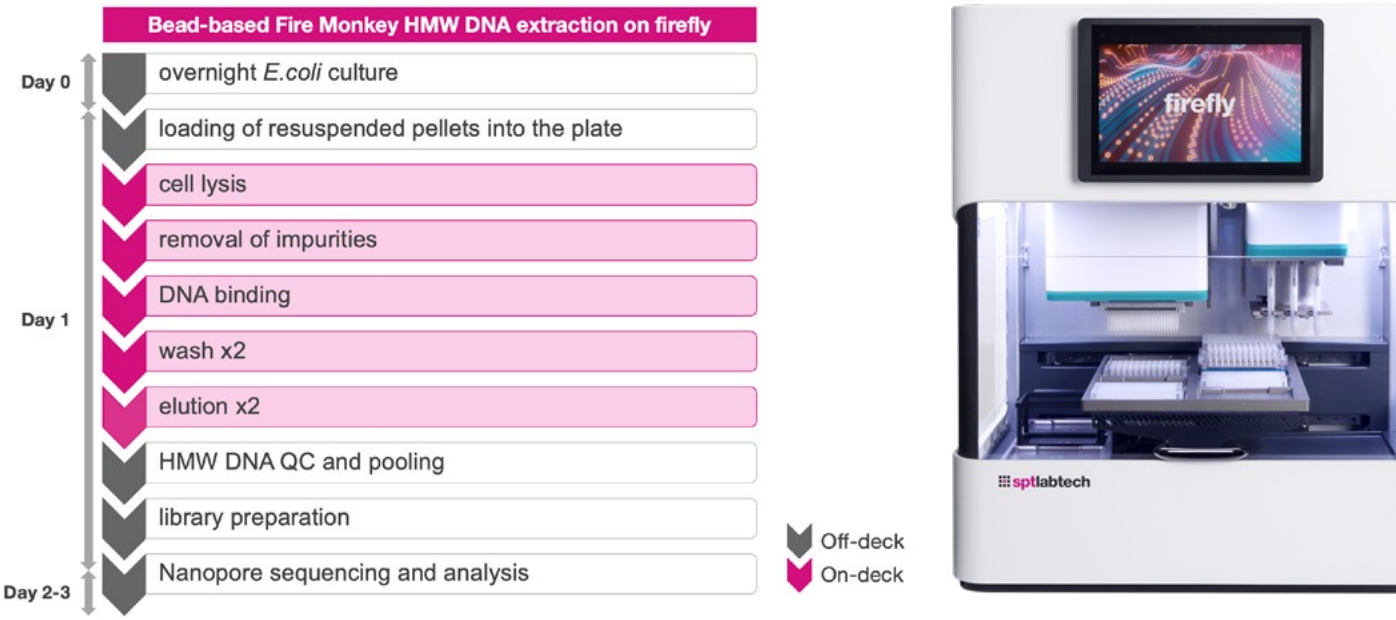

Figure 1: Schematic workflow of automated bead-based HMW DNA extraction using the Fire Monkey kit.

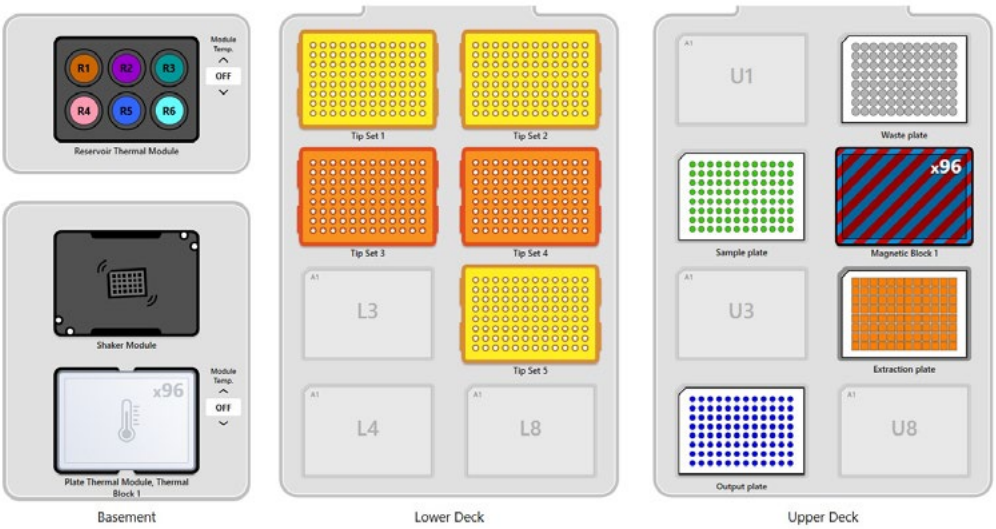

Figure 2: Starting firefly deck layout for automated Fire Monkey bead-based HMW DNA extraction.

results

performance evaluation of automated extraction

Table 1. DNA fragment length and yields obtained after manual (CTRL) and firefly-assisted (A1-A8) extraction.

		Femto Pulse			
Sample	**Elution volume (µL)**	**Average strand length (kb)**	**Molecular ratio (30kb cut-off)***	**Concentration (ng/µL)**	**Total yield (µg)**
A1	14 + 14	156.4	2	119	3.32
A2	14 + 14	149.2	N/A^	98	2.74
A3	14 + 14	174.8	2.4	132	3.69
A4	14 + 14	168.6	3	127	3.55
A5	14 + 14	191.4	3	115	3.22
A6	14 + 14	170.7	1.8	105	2.94
A7	14 + 14	163.6	1.5	138	3.86
A8	14 + 14	174.2	2	135	3.78
CTRL	20 + 20	125.7	1.27	88	3.52

*The molecular ratio is calculated by dividing the number of molecules above a certain cut-off to the molecules below. For instance, the molecular ratio of 2 for 30kb cut-off indicates that there are twice as many molecules larger than 30kb as smaller fragments.

^No fragments smaller than 30kb were observed.

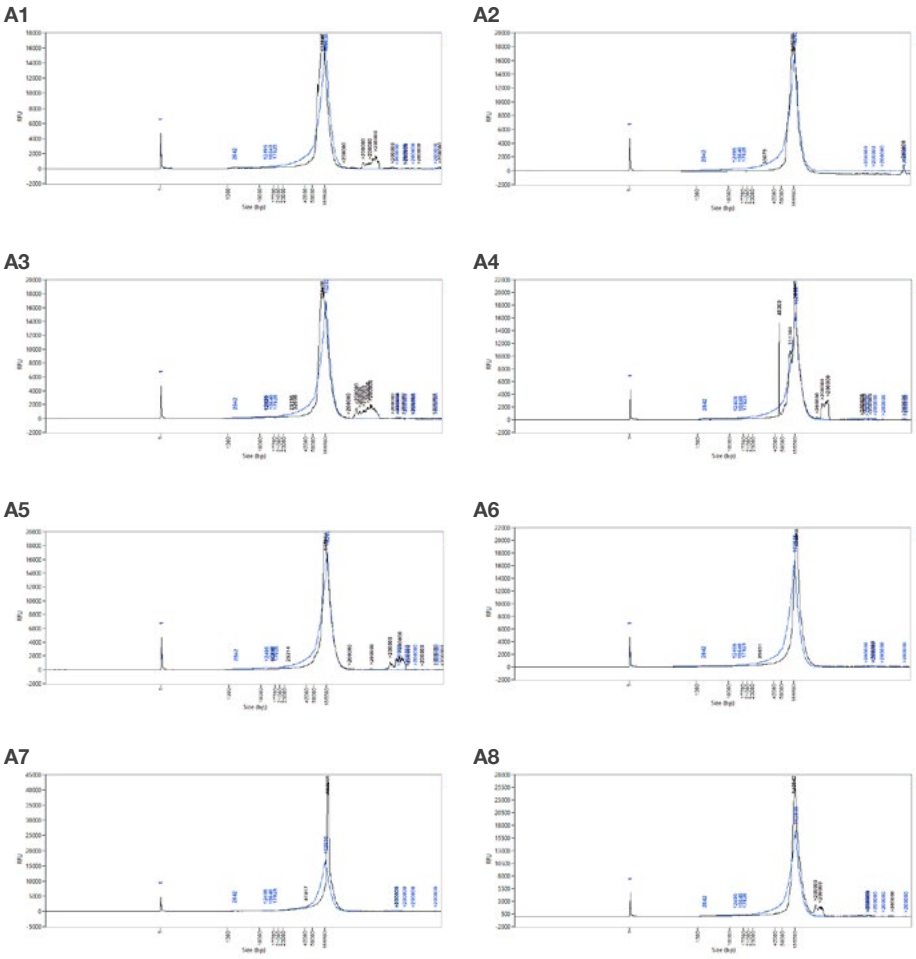

Figure 3: Femto Pulse traces for firefly samples (black) and pooled CTRL samples (blue). The HMW DNA extracted on firefly using Fire Monkey technology is highly enriched for molecules larger than 100kb. (RFU = relative fluorescence units).

Nanopore sequencing data

Table 2. Single run Nanopore sequencing data collected at different timepoints.

	CTRL 24 hrs	firefly 23 hrs*	firefly 46 hrs
total bases- raw data (Gb)	11.94	7.2	12.61
reads generated	1,560,000	890,620	1,720,000
read length N50 (kb)	20.55	35.78	34.06

*Nuclease flush was performed at 23 hrs and the flow cell was re-loaded.

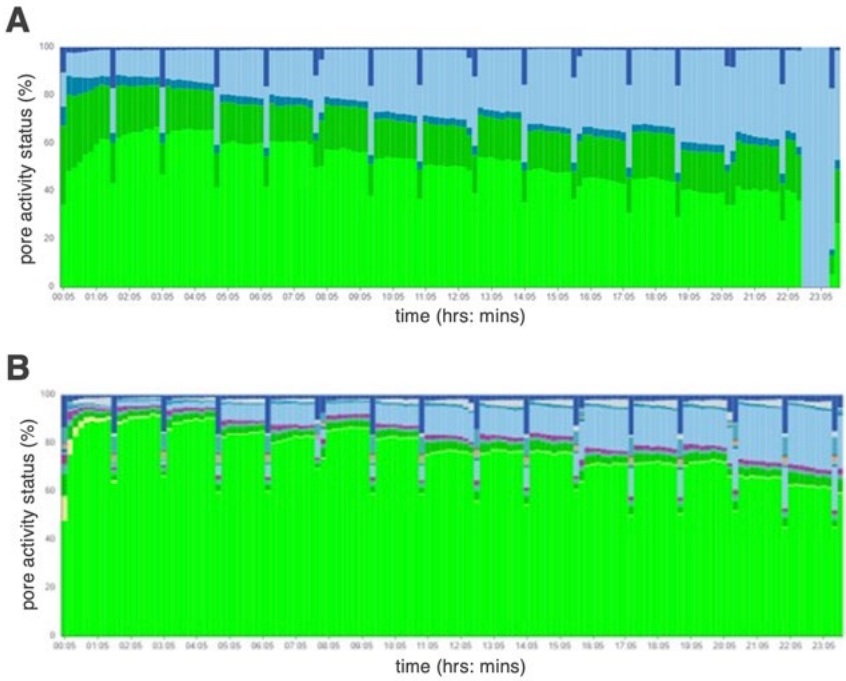

Figure 4: Both firefly (A) and CTRL (B) samples show a good pore occupancy during first 23 hrs of sequencing, indicating good quality libraries.

summary

- Combining the bead-based RevoluGen Fire Monkey chemistry with firefly liquid handling technology enables the rapid extraction of HMW DNA of sufficient yield and quality for Nanopore long-read sequencing.
- The demonstrated approach significantly reduces hands-on time and eliminates the requirement for special vacuum manifolds or plate centrifuges, multiple lysate transfer steps and on-deck storage of large volumes of reagents.
- This automated workflow is robust as shown by the low %CV between replicates. CV of 7.6% was observed for the average fragment size distribution (range: 149kb-191kb) and 12% for HMW DNA yields (range: 2.7-3.9 µg) (Table 1 and Figure 3).
- While the Nanopore library prepared from the pooled firefly samples generated fewer total bases (7.2Gb vs 11.94Gb), it delivered higher read length N50 (35.78kb vs 20.55kb) than the manual CTRL sample (Table 2).
- The same DNA extract could be used for short- and hybrid (short/long)-read workflows, as already shown for samples extracted with Fire Monkey spin column kit[1,2], providing users with a unique one stop sequencing-agnostic automated platform.

references

1. Goodman RN, Tansirichaiya S, Brouwer MSM, Roberts AP. Intracellular Transposition of Mobile Genetic Elements Associated with the Colistin Resistance Gene *mcr-1*. Microbiology Spectrum. 2023;11(1).
2. Carter C, Hutchison AA, Rudder S, et al. Uropathogenic *Escherichia coli* population structure and antimicrobial susceptibility in Norfolk, UK. Journal of Antimicrobial Chemotherapy. 2023;78(8):2028-2036.

REVOLUGEN | sptlabtech