

Application Note – innuPREP SE UHMW DNA Blood & Cells Kit-PP Mini

Automated extraction of UHMW DNA for Nanopore sequencing using PurePrep Mini

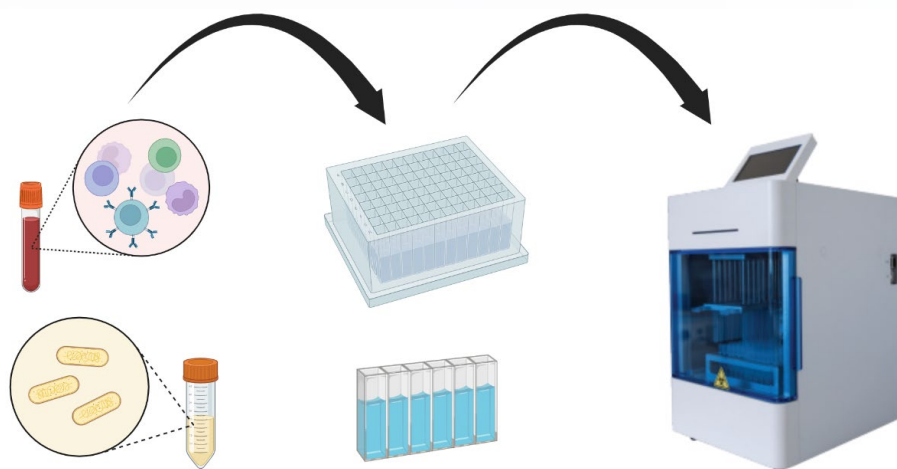
Short Product Description

Extraction of ultra-high molecular weight (UHMW) DNA is crucial for molecular applications such as long-read sequencing, *de novo* sequencing, genome assembly, and optical mapping. UHMW DNA offers a more complete view of the genome that is otherwise not possible with conventional next-generation sequencing. It is, however, very difficult to extract and isolate UHMW DNA that is highly pure and highly intact. Most currently used technique for extracting UHMW DNA are either challenging or expensive, especially when automated DNA extraction technologies are involved. The PurePrep Mini system is an easy and economical option for automated extraction and purification of UHMW DNA using innuPREP SE UHMW DNA Blood & Cells Kit-PP Mini.

The kit is based on the patented SmartExtraction Technology using Smart Modified Surfaces invented by IST Innuscreen GmbH. The extraction method relies on the adsorption of genomic DNA (gDNA) to Smart Modified Surfaces of the modified PurePrep Tip Comb and requires no magnetic particles for DNA binding. After washing, gDNA is eluted from the Smart Modified Surfaces and is of excellent quality needed for long-read sequencing technologies.

The PurePrep Mini is a remarkably compact benchtop device specialized in DNA and RNA extraction and purification. Ideally suited for small-scale laboratories, the PurePrep Mini allows simultaneous processing of up to 16 samples.

Workflow: Automated sample lysis and DNA extraction with the PurePrep Mini device



Automated extraction workflow on PurePrep Mini device:

1. Sample lysis
2. DNA binding
3. DNA washing
4. DNA dissolving

Total Time: < 2.5h

Example of Application

A. Extraction of DNA from whole blood samples

Starting material: 2 ml whole blood

Lysis of erythrocytes and pelleting of PBMC's. Resuspension of the PBMC pellet and automated DNA extraction on PurePrep Mini device (lysis-binding-washing-dissolving). Samples were eluted in 400 µl elution buffer.

B. Results

1. Confirmation of yield and quality of the extracted UHMW DNA

To determine the DNA yield and quality, 1 µl DNA was analysed on the Agilent 4150 TapeStation System (Agilent) and used for spectrophotometric measurements. The results indicate that the majority of the DNA is longer than 60,000 bp and shows a high integrity. The DNA yield is very high and the ratios 260/280 and 260/230 are excellent.

Table 1. Spectrophotometric measurements of UHMW DNA extracted from human whole blood.

Sample name	Concentration (ng/µl)	DNA yield (µg)	A _{260/280}	A _{260/230}
Whole Blood	130	39.0	1.82	2.27

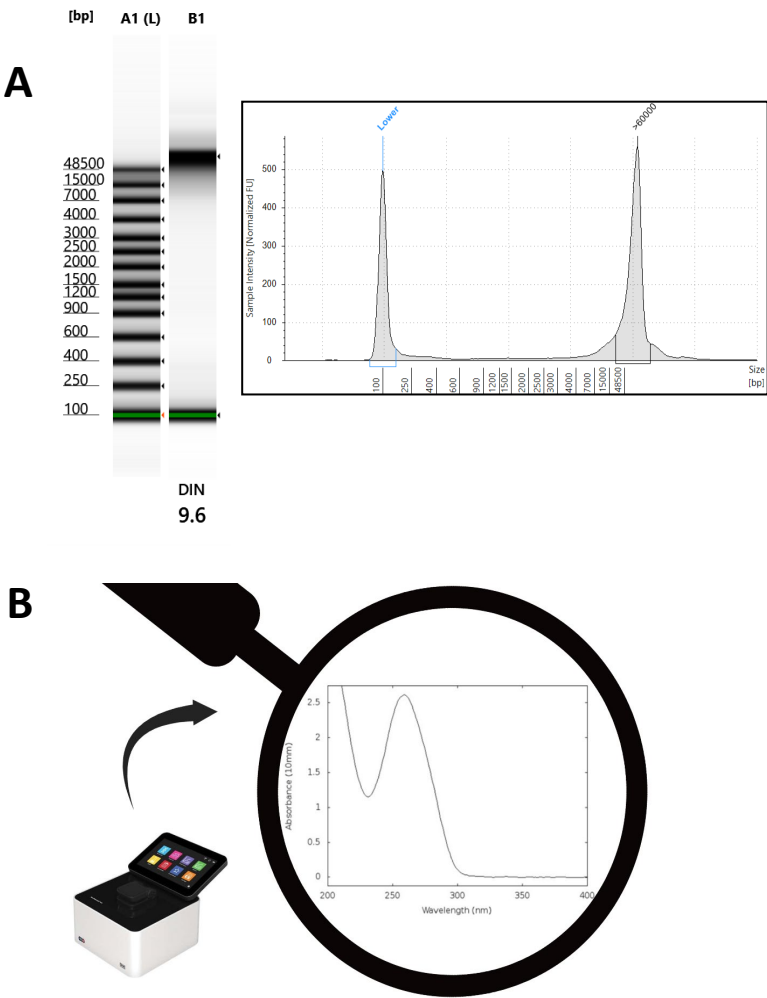


Figure 1. (A) Agilent 4150 TapeStation analysis (B) Spectrophotometrical analysis

2. Ultra-Long DNA sequencing using SQL-VLK114; Oxford Nanopore Technologies

20 µg of extracted DNA was used for long-read sequencing. After library preparation and purification the DNA was loaded on the SpotON™ Flow Cell onto MinION Mk1B (Oxford Nanopore Technologies) and data acquisition was done using MinkNOW v22.12.7 software.

Results of the Nanopore sequencing showed that the UHMW DNA extracted from human whole blood generated high-quality reads and very good throughput (Table 2).

Table 2. Oxford Nanopore sequencing run summary

Run summary

DATA OUTPUT

Estimated bases

8.76 Gb

Data produced

94.67 GB

Reads generated

344.29 k

Estimated N50

53.75 kb

BASECALLING

Reads called

99.96%

Bases called (min Q score: 8)

6.64 Gb

1.51 Gb

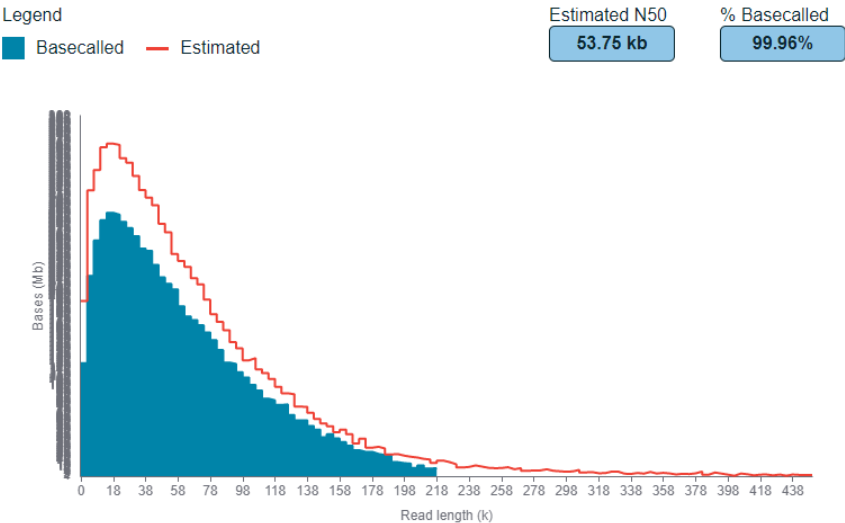
Pass

Fail

Sequence output

READ LENGTHS · OUTLIERS REMOVED

The read length graph shows the total number of bases vs the read length. The longest 1% of strands are classified as outliers, and excluded to allow focus on the main body of data.



OUTLIERS

The longest 1% of strands are classified as outliers, and aggregated into groups to show their relative amounts.

Read length (kb)	Aggregated reads (Mb)
220 - 284	59.38
284 - 348	6.13
348 - 412	0.36
412 - 476	None
476 - 484	0.49

Conclusion

The results show that the innuPREP SE UHMW DNA Blood & Cells Kit – PP Mini was able to successfully extract UHMW DNA from human whole blood using the PurePrep Mini system. The isolated UHMW DNA could be successfully used for long-read sequencing on Oxford Nanopore Technologies device.