

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

### Automated extraction of UHMW DNA for Nanopore sequencing using PurePrep Mini

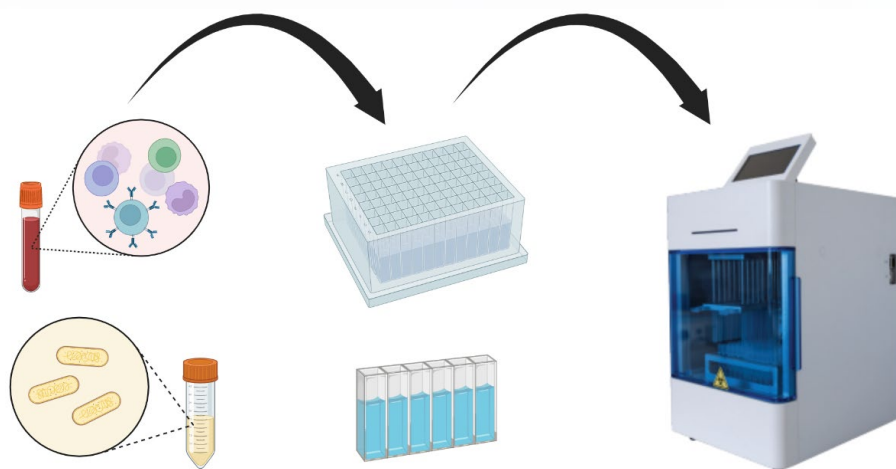
#### Short Product Description

Extraction of ultra-high molecular weight (UHMW) DNA is crucial for molecular applications that involved the use of very long DNA molecules, among which include 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 a typical 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. An easier and more economical option for automated extraction and purification of UHMW DNA would be through the PurePrep Mini system 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 required no magnetic particles for DNA binding. After washing, gDNA is eluted from the Smart Modified Surfaces and from 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 for small-scale laboratories. The PurePrep Mini allows simultaneous processing of up to 16 samples.

#### Workflow: Complete 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  $\mu$ l elution buffer and transferred to clean 1.5 ml tubes.

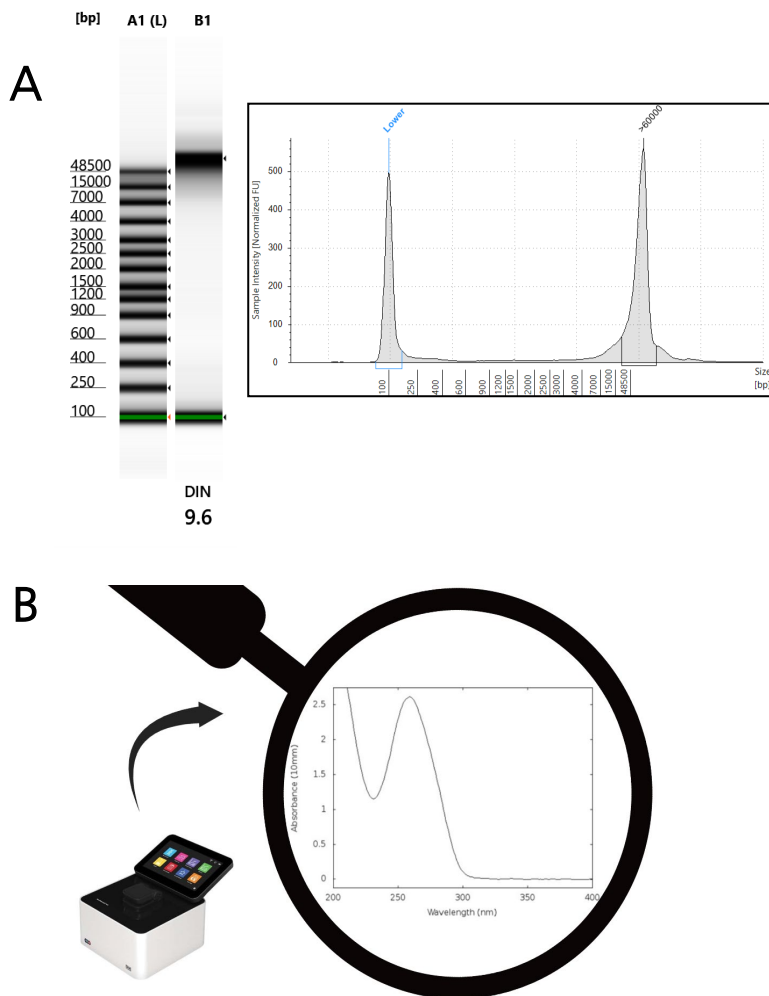
## B. Results

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

To determine the DNA yield and quality, 1  $\mu$ l DNA was used on 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 between 260/280 and 260/230 are excellent.

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

Sample name	Concentration (ng/ $\mu$ l)	DNA yield ( $\mu$ g)	A <sub>260/280</sub>	A <sub>260/230</sub>
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

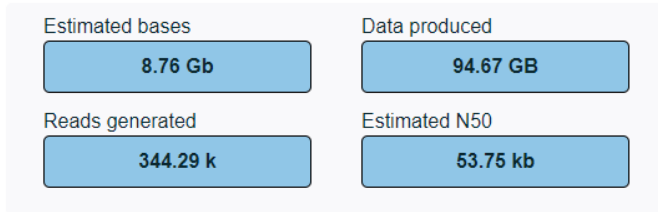
20 µg of extracted DNA was used for the long-read sequencing reaction. After library preparation and purification the DNA was loaded on the SpotON™ Flow Cell on MinION Mk1B (Oxford Nanopore Technologies) and data acquisition was done using MinKNOW v22.12.7 software.

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).

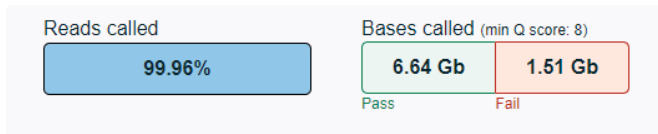
Table 2. Oxford Nanopore sequencing run summary

### ^ Run summary

#### DATA OUTPUT



#### BASECALLING



### ^ 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.

Legend

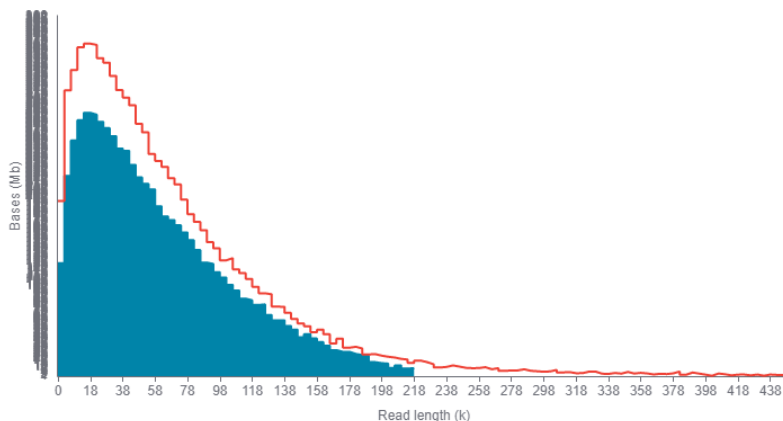
■ Basecalled — Estimated

Estimated N50

53.75 kb

% Basecalled

99.96%



#### ^ 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.