Supplemental Material of:

Krautwurst M, Eikhof A, Winkler S, Bross D, Kersten B, Müller NA (2024)

High-molecular-weight DNA extraction for broadleaved and conifer tree species. Silvae Genetica 73(1): 85 – 98

Figures

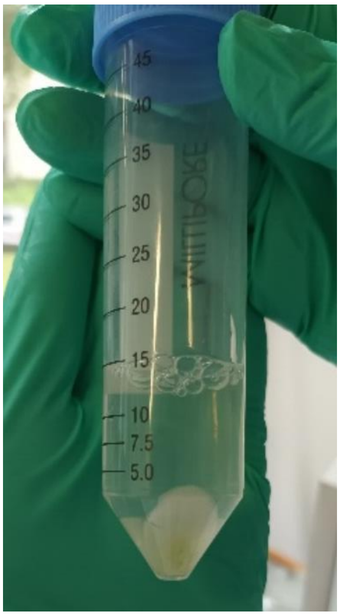

Figure S.1: *Taxus baccata* nuclei pellet in 50 ml tube in NIB wash buffer after last washing step with NIB.

Figure S.2.: *Taxus baccata* Nuclei pellet in 1.5 ml Eppendorf tube before nuclei extraction with Nanobind plant nuclei kit.

Figure S.3.: *Fraxinus excelsior* Nuclei pellet in 50 ml tube in NIB wash buffer after last washing step with NIB.

Figure S.4.: *Fraxinus excelsior* Nuclei pellet in 1.5 ml Eppendorf tube before nuclei extraction with Nanobind plant nuclei kit.

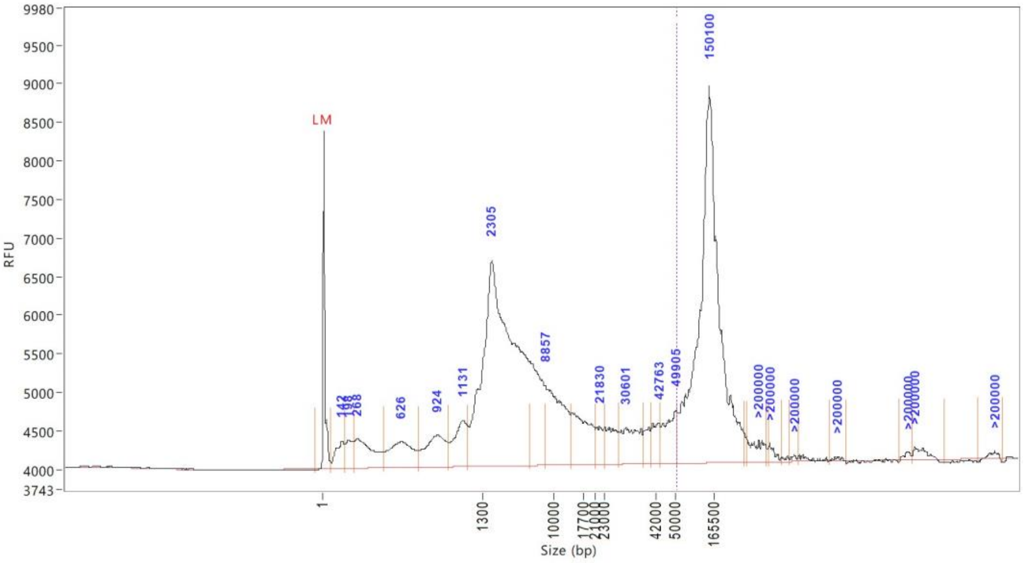

Figure S.5.: Femto Pulse DNA sizing QC analysis of *Taxus baccata* L. sample 1.

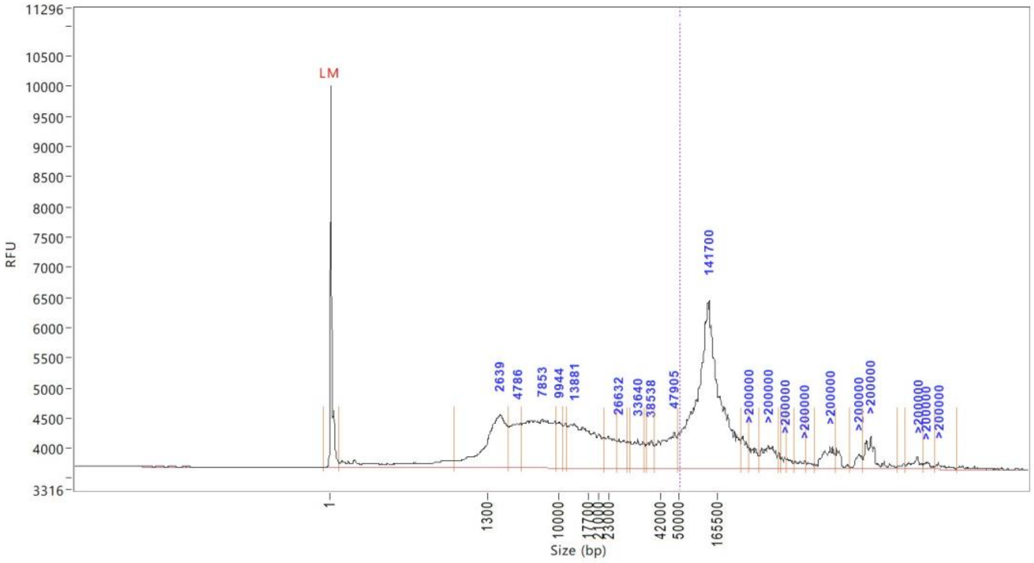

Figure S.6: Femto Pulse DNA sizing QC analysis of *Taxus baccata* L. sample 3.

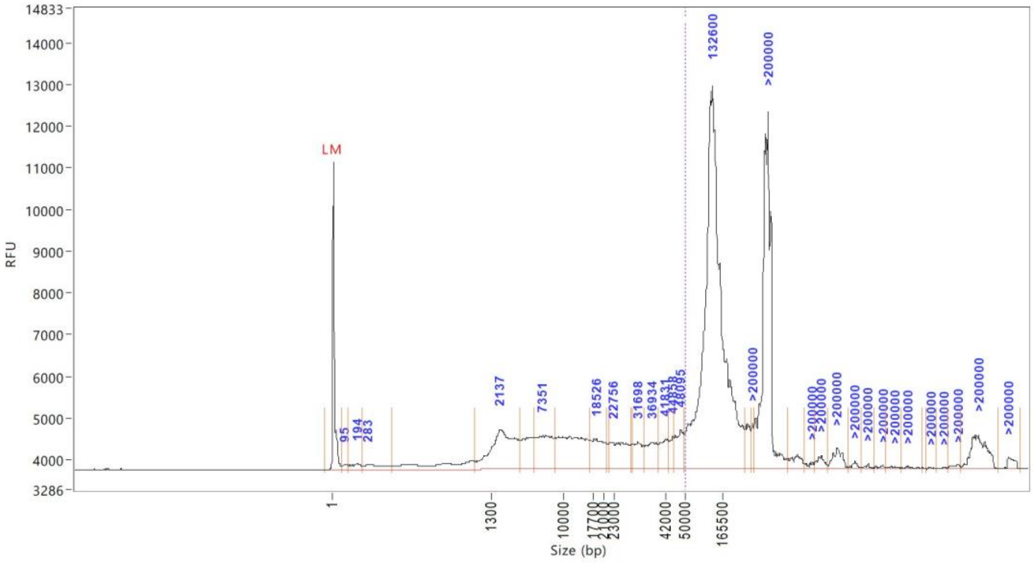

Figure S.7.: Femto Pulse DNA sizing QC analysis of *Taxus baccata* L. sample 4.

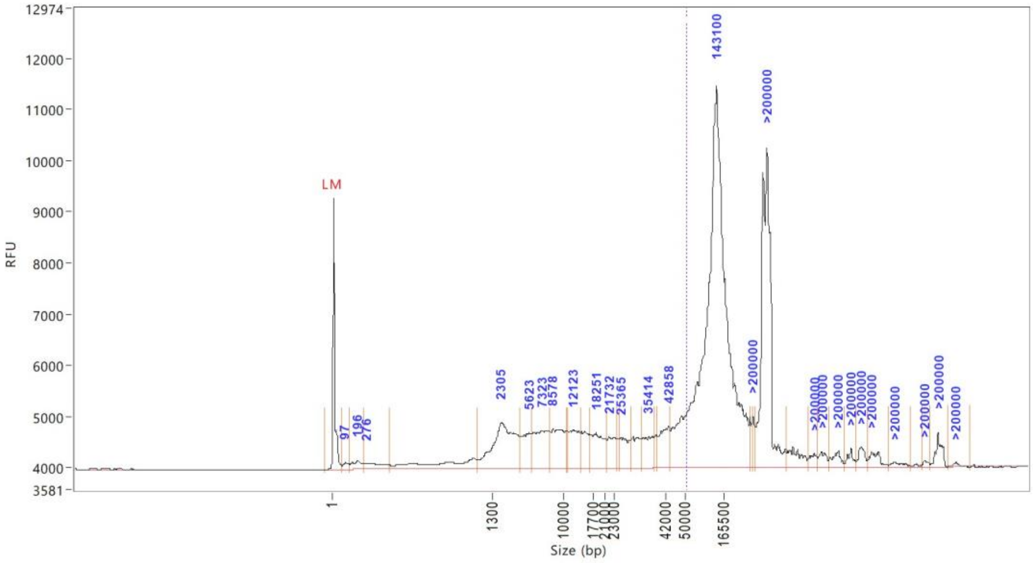

Figure S.8.: Femto Pulse DNA sizing QC analysis of *Taxus baccata* L. sample 5.

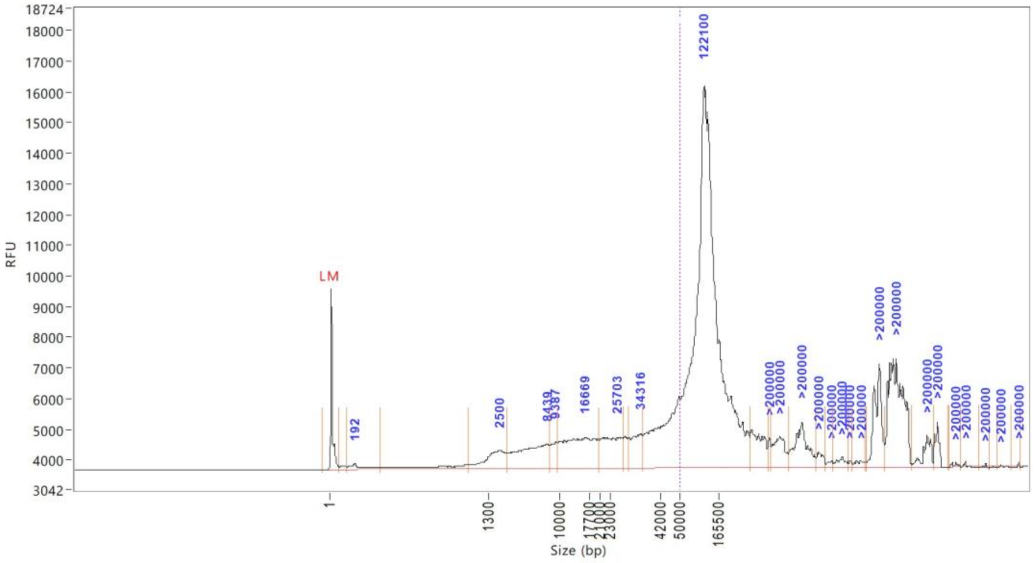

Figure S.9.: Femto Pulse DNA sizing QC analysis of *Taxus baccata* L. sample 6.

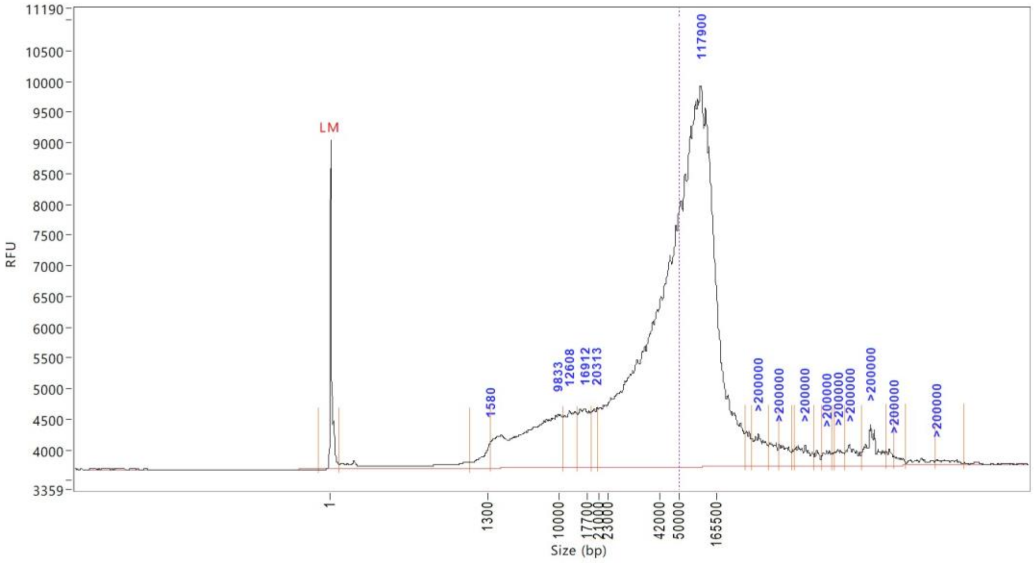

Figure S.10.: Femto Pulse DNA sizing QC analysis of *Taxus baccata* L. sample 7.

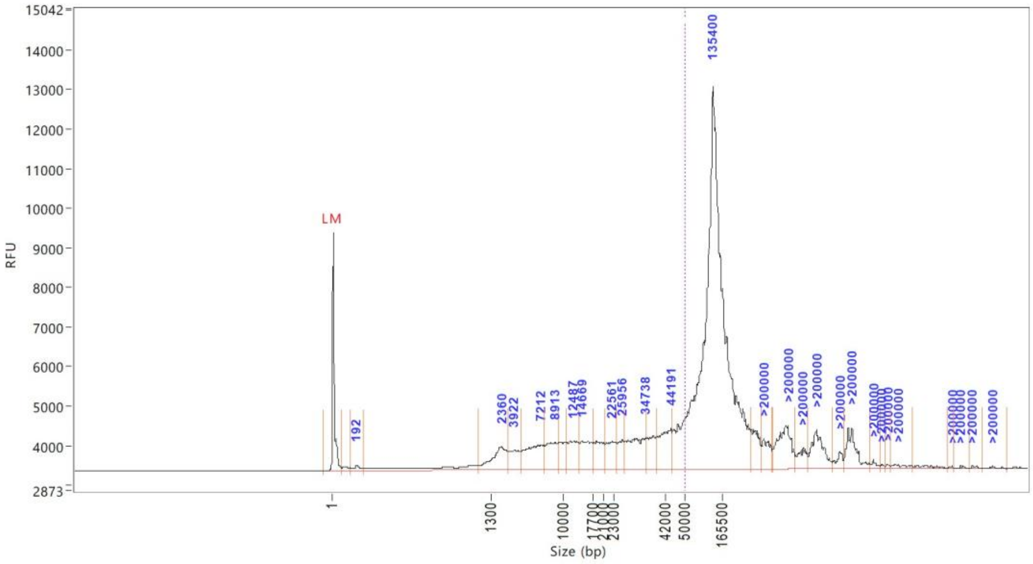

Figure S.11.: Femto Pulse DNA sizing QC analysis of *Taxus baccata* L. sample 8.

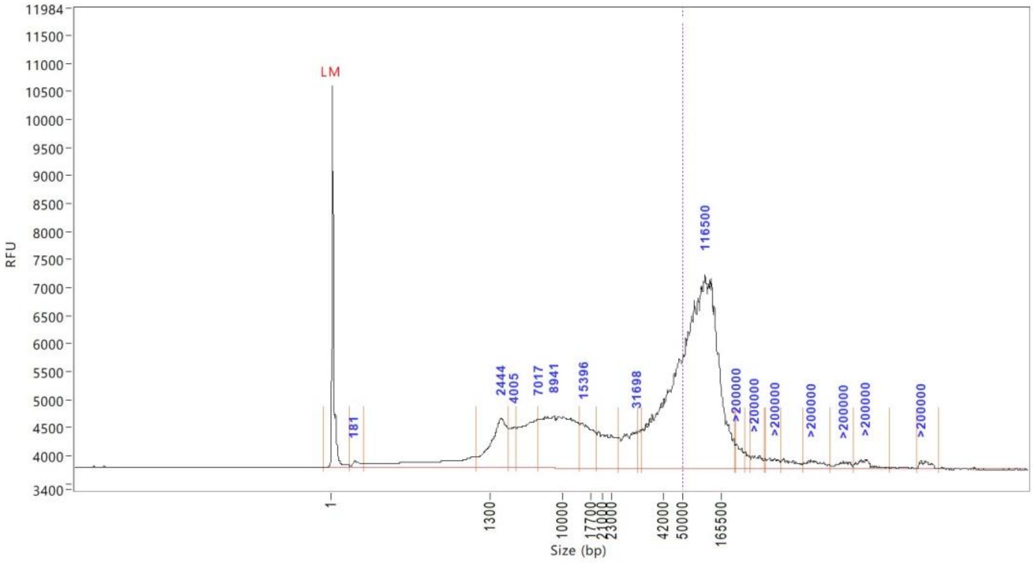

Figure S.12.: Femto Pulse DNA sizing QC analysis of *Taxus baccata* L. sample 9.

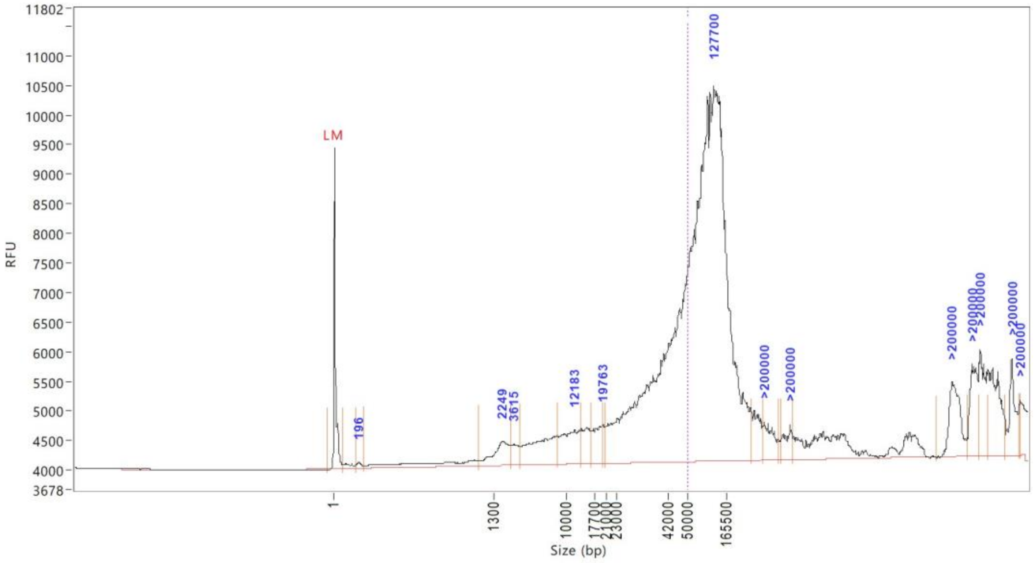

Figure S.13.: Femto Pulse DNA sizing QC analysis of *Taxus baccata* L. sample 10.

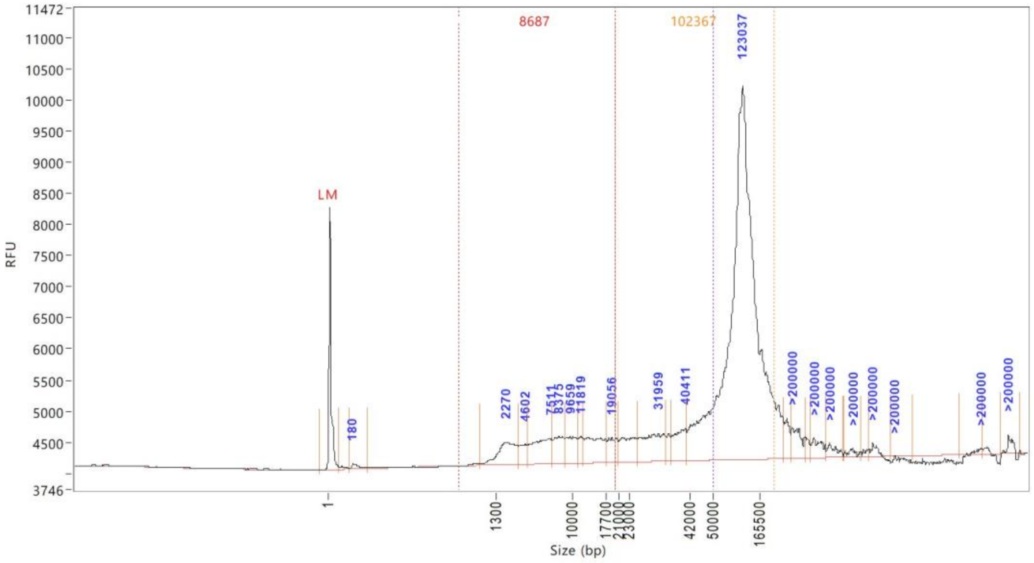

Figure S.14.: Femto Pulse DNA sizing QC analysis of *Taxus baccata* L. sample 11.

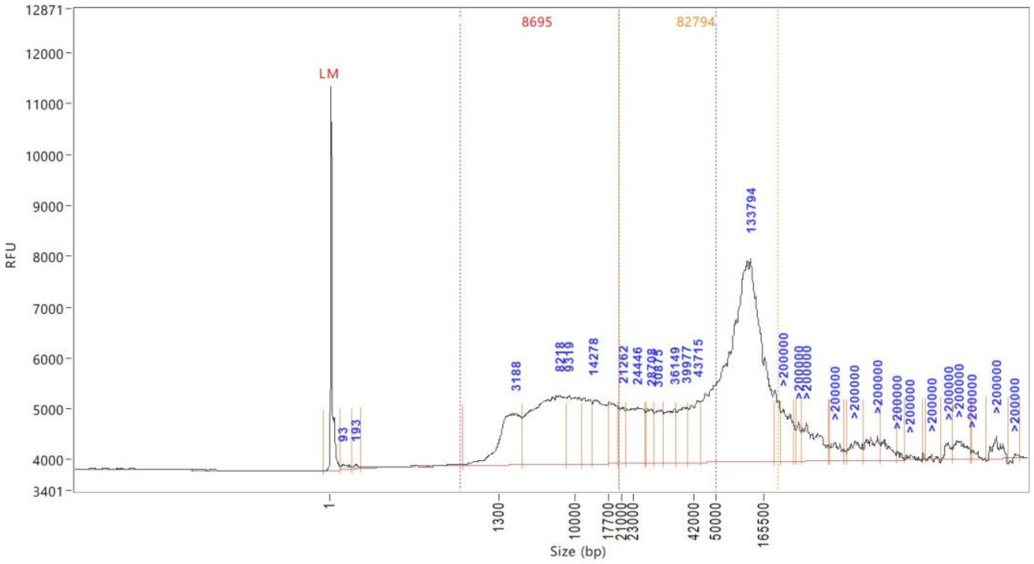

Figure S.15.: Femto Pulse DNA sizing QC analysis of *Taxus baccata* L. sample 12.

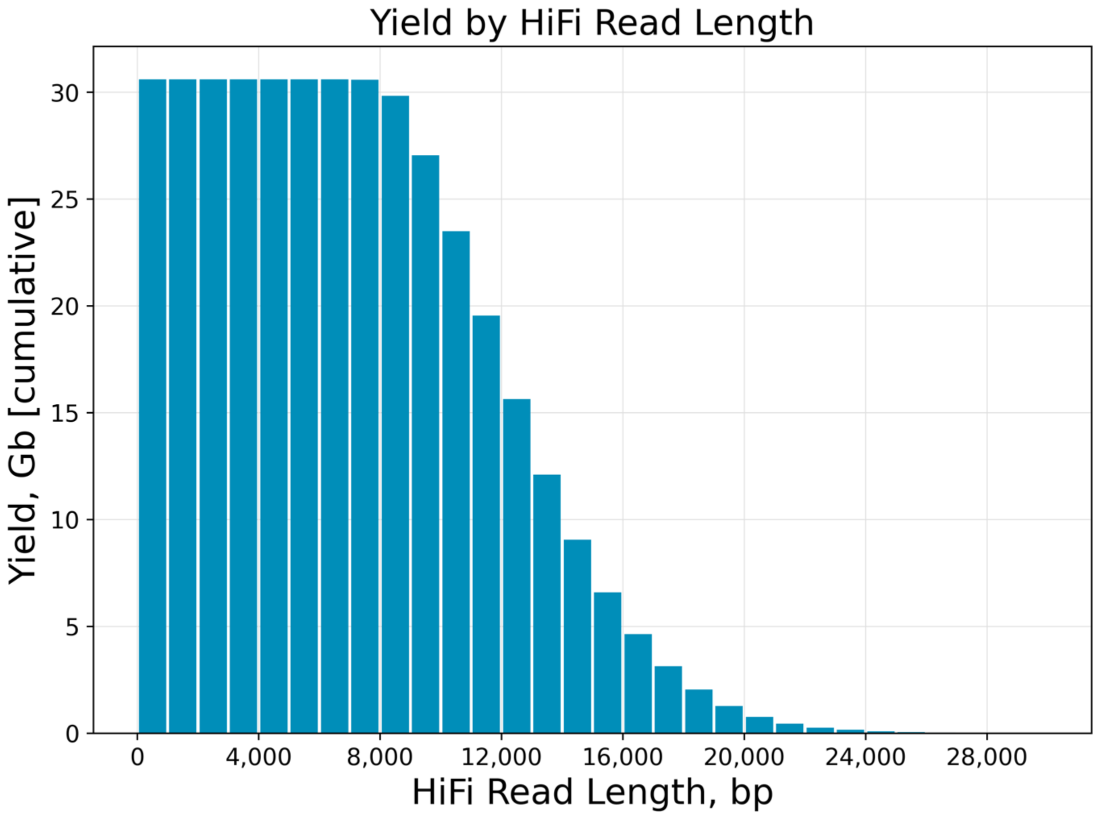

Figure S.16.: Sequencing results of *Taxus baccata* DNA using PacBio HiFi sequencing (Run ID 21, Table 8). The total yield of circular consensus sequencing (CCS) reads of this SMRT cell was 30.6 Gb. These CCS reads have been called with the PacBio SMRT link pipeline (SMRTlink version 11.0.0.146107)

Tables

Table S.1: High-molecular-weight DNA extraction protocol steps of *Faxinus excelsior*.

sample	**Grinding**	**Washing steps (NIB), Isolation**	**Sorbitol wash**	**Washing steps (NIB), Extraction**	**Lysation**	**Centrifugation force**
I	TissueRuptor	4	1	4	2 h	5000 x g
II	TissueRuptor	4	1	4	2 h	5000 x g
III	Mortal + Pestel	4	1	4	2 h	7000 x g
VI	TissueRuptor	4	1	4	2 h	5000 x g

Table S.2: High-molecular-weight DNA extraction protocol steps of *Taxus baccata*.

sample	**Grinding**	**Washing steps (NIB), Isolation**	**Sorbitol wash**	**Washing steps (NIB), Extraction**	**Lysation**	**Centrifugation force**
1	Mortal + Pestel	1	2	4	1 h	3000 x g
2	Mortal + Pestel	1	2	4	1.15 h	3000 x g
3	Mortal + Pestel	1	2	4	1.15 h	3000 x g
4	Mortal + Pestel	1	2	4	1.30 h	3000 x g
5	Mortal + Pestel	1	2	4	1.30 h	3000 x g
6	Mortal + Pestel	1	2	4	1 h	3000 x g
7	Mortal + Pestel	1	2	4	1 h	3000 x g
8	Mortal + Pestel	1	2	4	1.15 h	3000 x g
9	Mortal + Pestel	1	2	4	1.15 h	3000 x g
10	Mortal + Pestel	1	2	4	1 h	3000 x g
11	Mortal + Pestel	1	2	4	1 h	3000 x g
12	Mortal + Pestel	1	2	4	1.20 h	3000 x g

Table S.3: High-molecular-weight DNA extraction quality and quantity values after applying the protocol to *Fraxinus excelsior*. For each measurement, 1 µl DNA was used. The green-marked rows are the samples that were used for ONT Sequencing.

	Before Short Read Eliminator				**After Short Read Eliminator**			
sample	**Nanodrop (ng/µl)**	**A260/A280**	**A260/A230**	**Qbit (ng/µl)**	**Nanodrop (ng/µl)**	**A260/280**	**A260/A230**	**Qbit (ng/µl)**
1	127.7	1.61	1.31	61.4	33.6	1.26	0.55	41.0
2	359.9	1.76	1.59	149	162.3	1.43	0.99	87.0
3	912.3	1.58	1.13	314.0	174.1	1.63	1.45	131.0
4	199.00	1.67	1.39	122	59.5	1.61	1.40	83.8
5	143	1.78	1.68	250	56.9	1.76	1.88	69.2
6	217.4	1.81	1.64	330	36.3	1.92	2.12	424

6 different HMW DNA preparations were considered for *Fraxinus excelsior*. HMW DNA extraction from *Fraxinus excelsior* is indicated for sequencing with Oxford Nanopore.