Supplementary Data

Table S1: Full bioinformatic analysis of sequencing data comparing the average of the three replicates for each of the sequencing set-ups to determine the enrichment factors based on the average of the three standard sequencing replicates for NipWT and compared to the standard promethION™ for NipGE.

Table S2: Overview of recommend conditions for either of the AS modes.

AS modes	Recommended conditions
Enrichment	- Target of interest present at low abundance in the mixture - Prior knowledge available on the target(s) of interest, including genetic fingerprint fully known and characterized
Depletion	- Highly abundant unwanted DNA from the matrix - Information on the unwanted matrix ingredients - No specific information on the target(s) of interest
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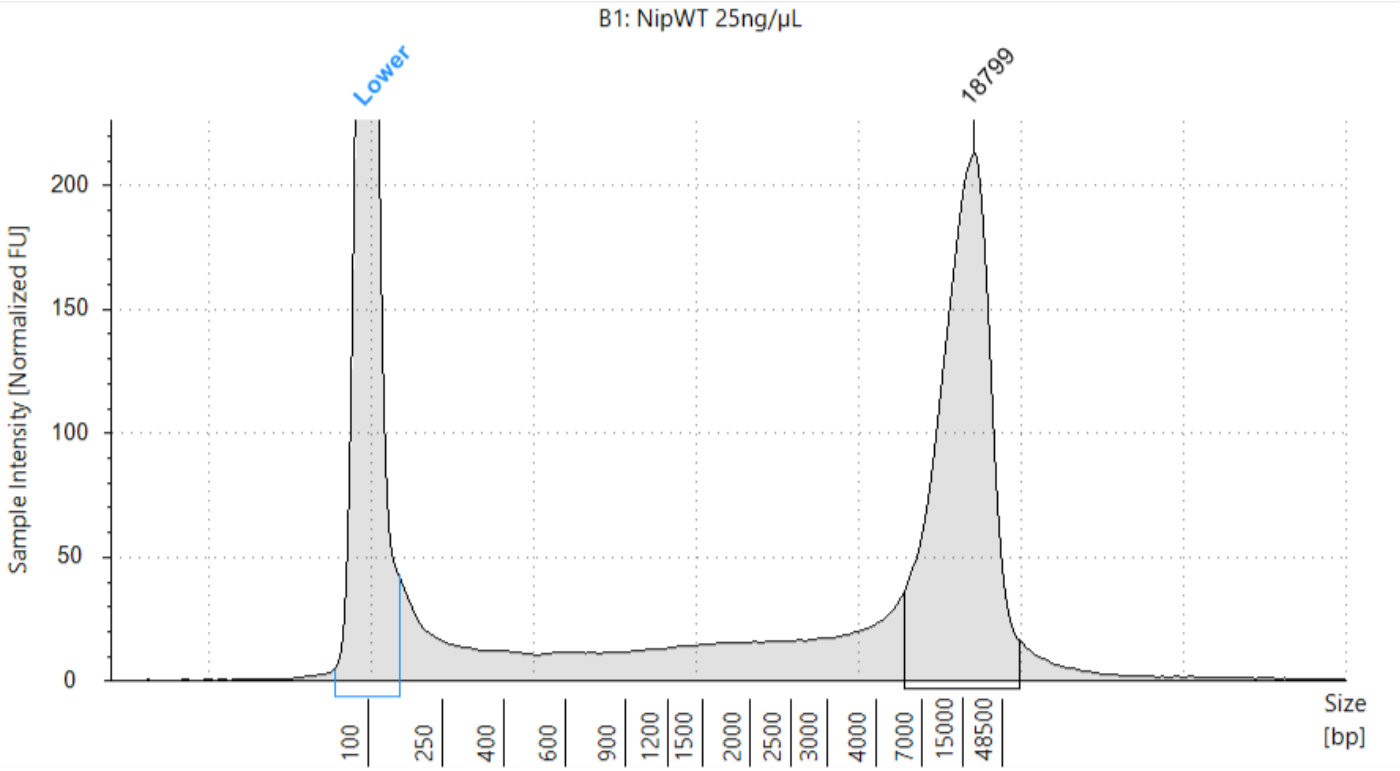
Table S3: full list of read depths for all the SNVs related to the cultivar specific marker of NipWT and NipGE for each of the sequencing setups. For each SNV the chromosome ID and position are shown together with the read depth and the reads for either the reference or alternative allele. In all of the cases, there were no reads for the reference allele, indeed confirming the presence of the SNV.

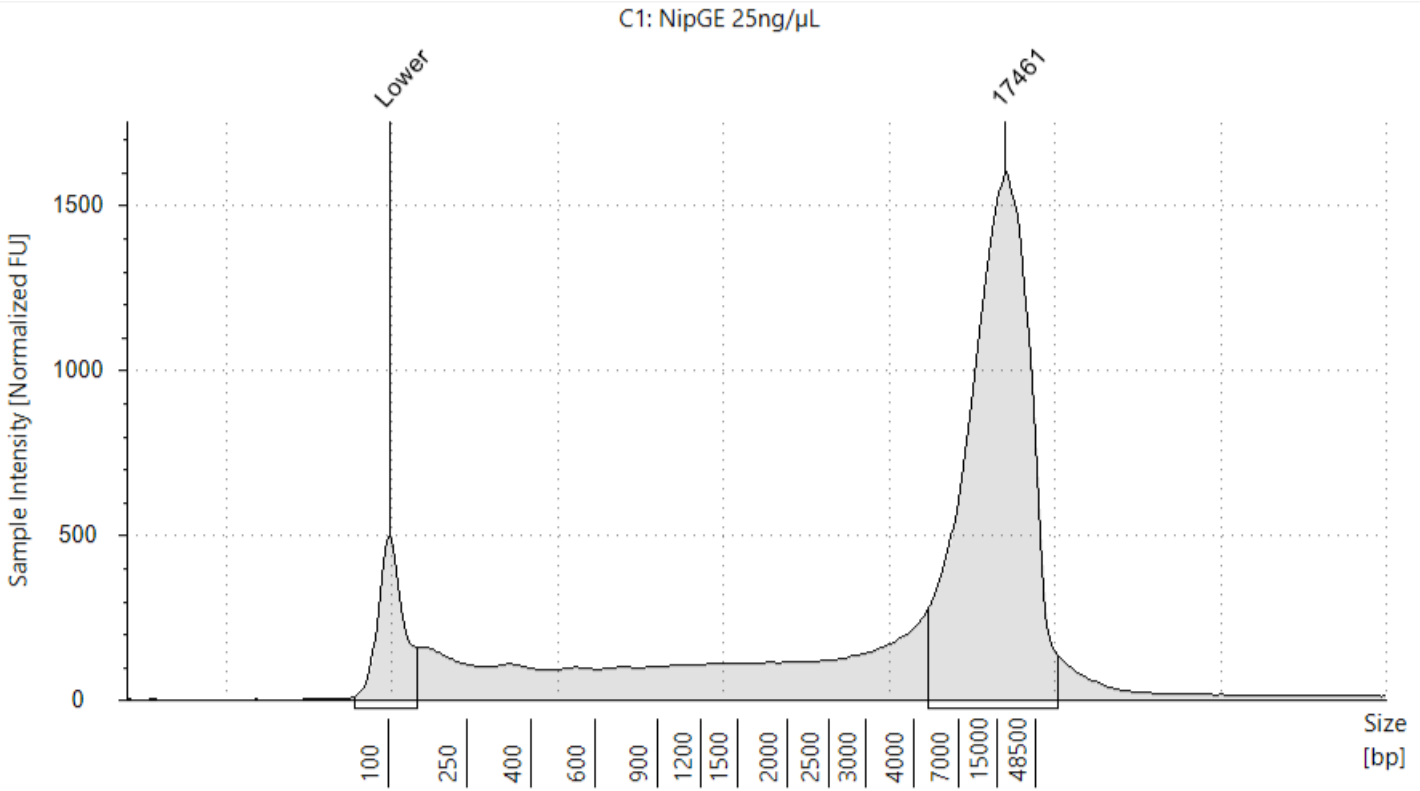
Table S4: Ratio verification of each DNA mixture using ddPCR. The ratio is calculated based on the total genome length of each crop species times the measured amount of copies to ensure that the ratio incorporates the smaller length of the rice haploid (Arumuganathan & Earle, 1991; Shang et al., 2023).

		Copies per reaction (20 µl)		Genome size (Mbp)	Total bases (Mbp)	
Mixture	Composition	Expected	Observed		Expected	Observed
1% NipWT + 99% Soybean	NipWT rice	55	54	385.7	21213.5	20719
	Soybean	2100	1860	1013.2	2127720	1884633
	Ratio				**0.99%**	**1.09%**
1% NipGE + 99% Soybean	NipGE rice	55	63	385.7	21213.5	24376
	Soybean	2100	2463	1013.2	2127720	2495512
	Ratio				**0.99%**	**0.97%**

A: NipWT


B1: NipWT 25ng/µL
Lower
18799
Sample Intensity [Normalized FU]
200
150
100
50
0
100
250
400
600
900
1200
1500
2000
2500
3000
4000
7000
15000
48500
Size
[bp]

B: NipGE


C1: NipGE 25ng/µL
Lower
17461
1500
1000
500
0
Sample Intensity [Normalized FU]
100
250
400
600
900
1200
1500
2000
2500
3000
4000
7000
15000
48500
Size
[bp]

C: Soybean

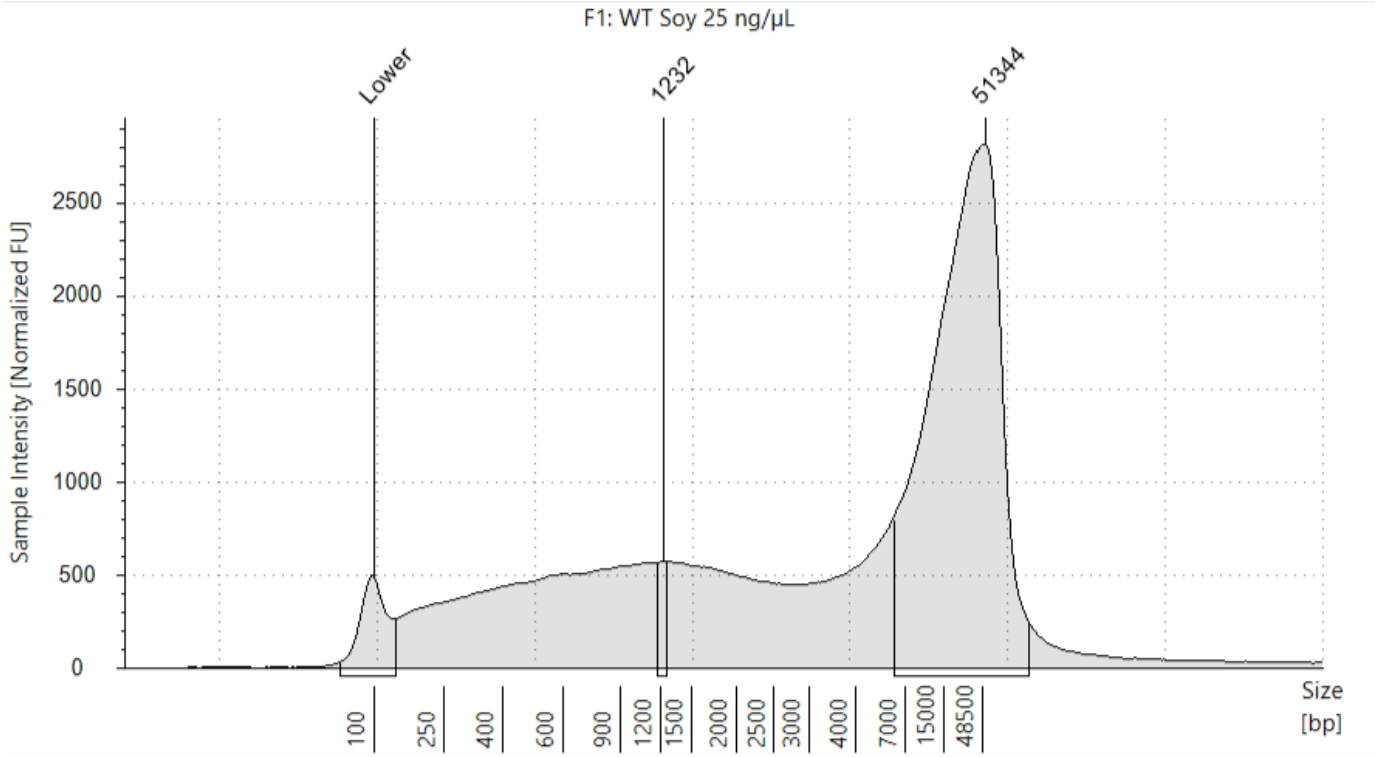
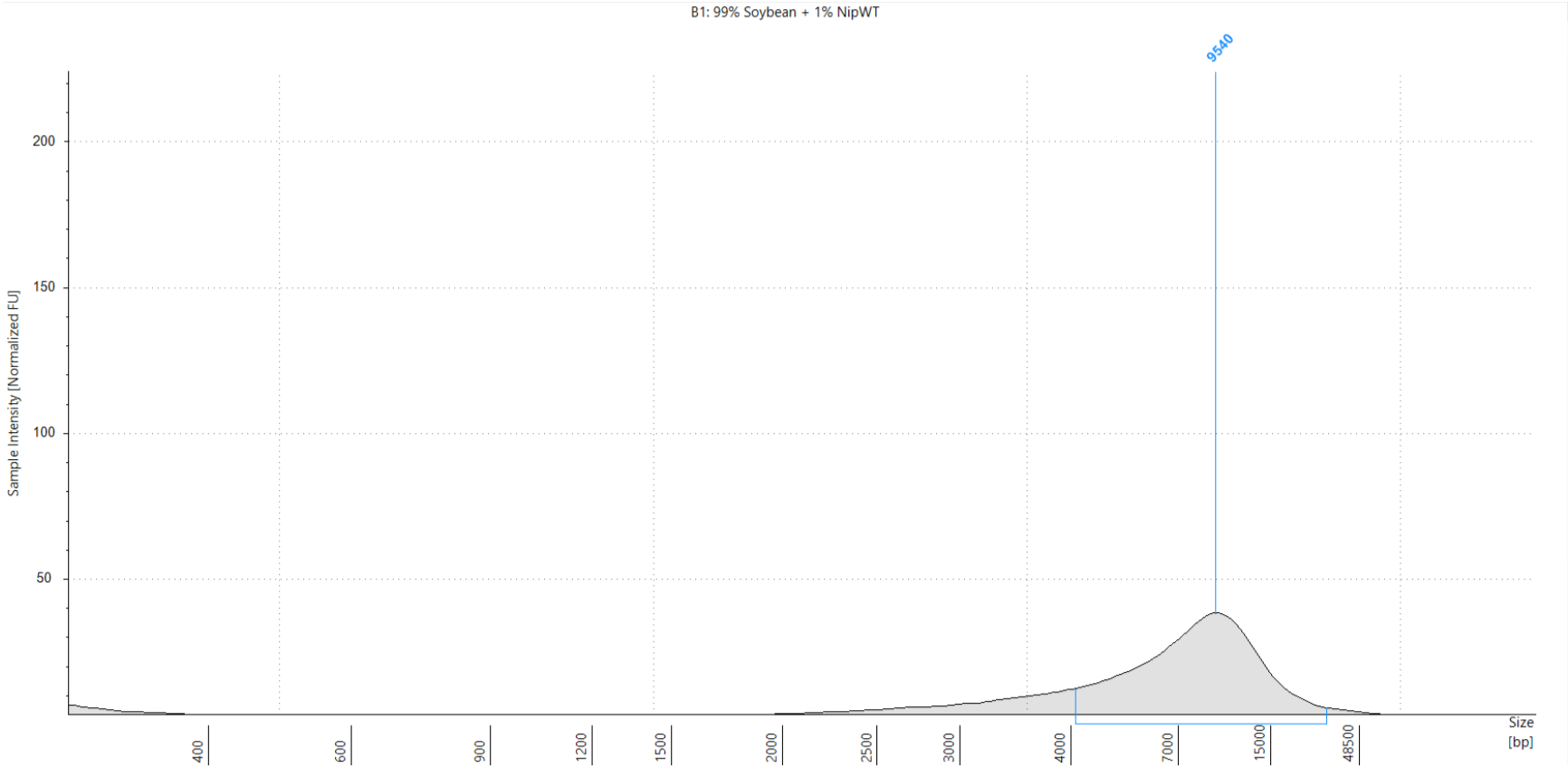


Figure S1: Genomic profiles of ingredients using a Tapestation 4200 device with the associated Genomic Screen Tape and reagents (Agilent Technologies, Santa Clara, California, USA). NipWT (A), NipGE (B) and Soybean (C).

A : 99% Soybean + 1% NipWT



B : 99% Soybean + 1% NipGE

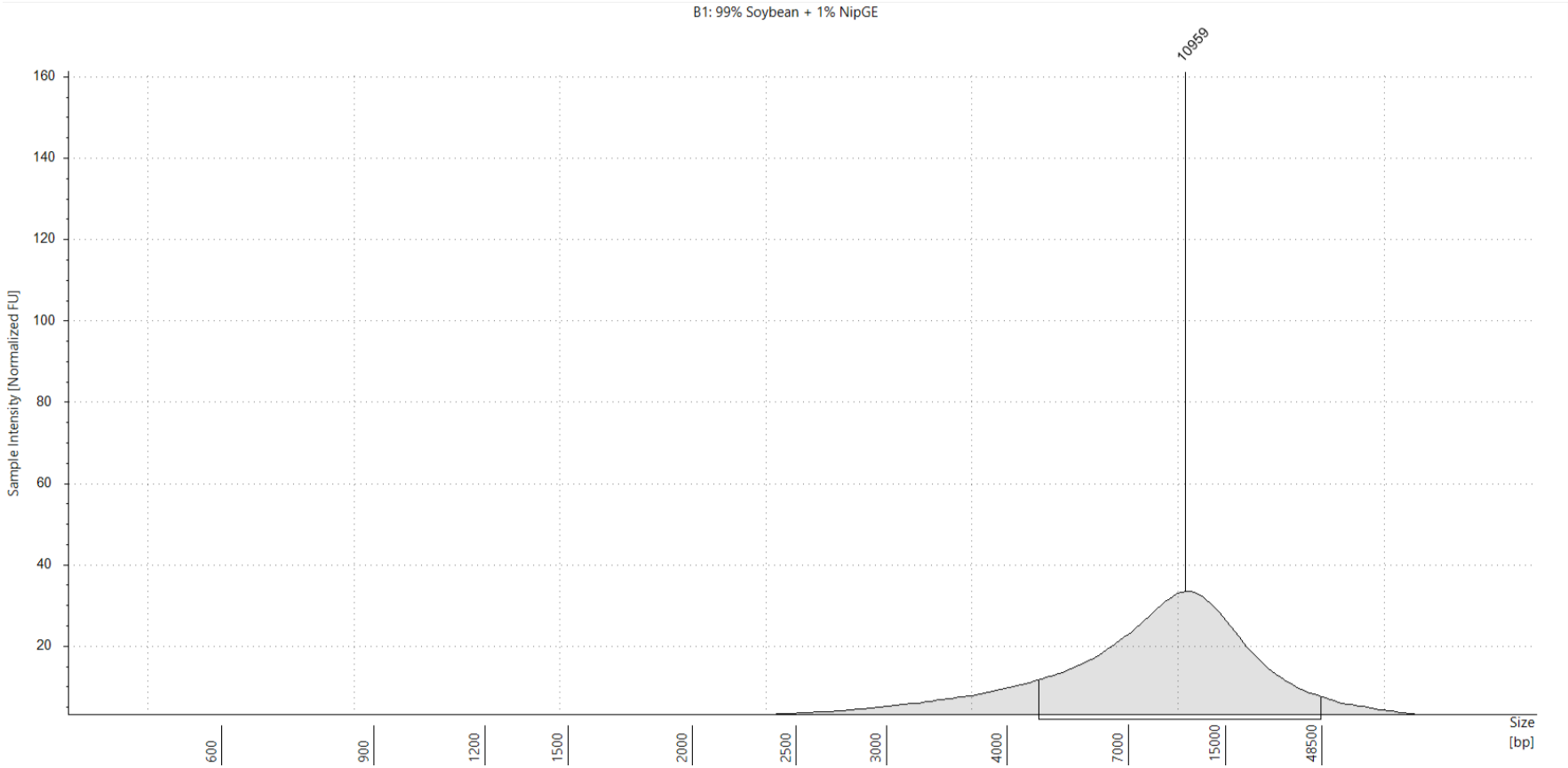


Figure S2: Verification of sequencing mix fragmentation length using a Tapestation 4200 device with the associated Genomic Screen Tape and reagents (Agilent Technologies, Santa Clara, California, USA). The ideal length of fragments for Adaptive sampling libraries is between 6 and 8 kb (Oxford Nanopore Technologies, 2020) for NipWT (A) and NipGE (B) mixture. The verification of the fragment length was performed directly after the fragmentation step, prior to further library preparation.

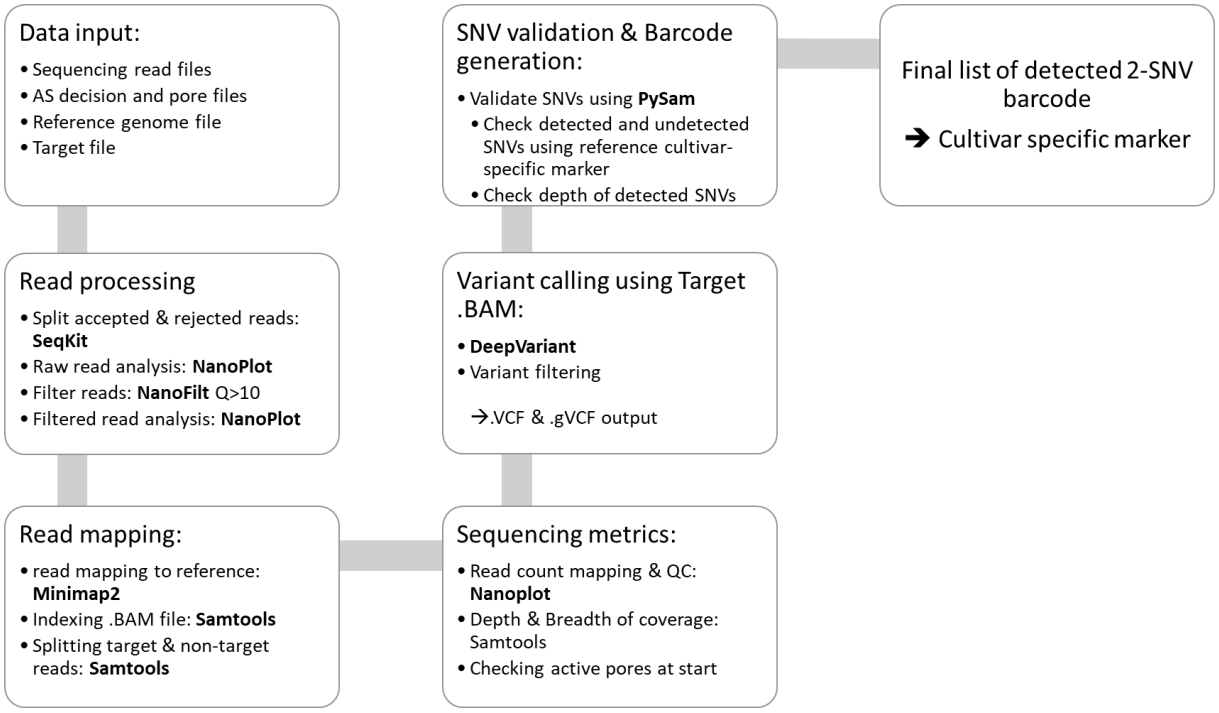


Figure S3: **Schematic overview of read processing pipeline from raw sequencing reads to cultivar-specific 2-SNV barcode generation**

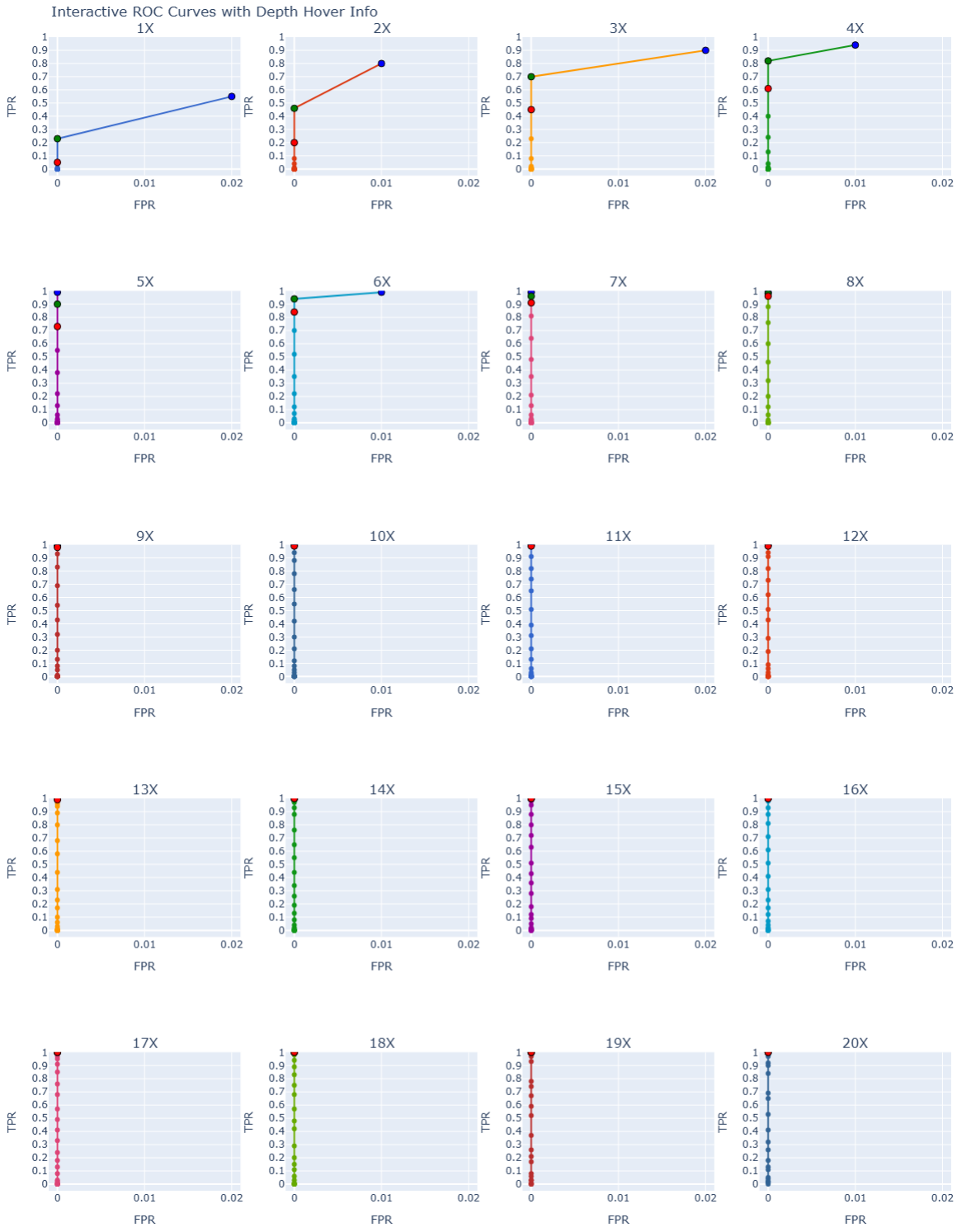


Figure S4: Receiver Operating Characteristic (ROC) curves for SNV detection within the Nipponbare-cultivar marker. Each subplot represents an average coverage depth, calculated as the mean true positive (TP) and false positive (FP) rates across replicates. Different points within each subplot indicate varying variant filtering cut-offs. The depth cut-offs of 1X, 2X, and 3X are shown in blue, green, and red, respectively, as the false positive rate (FPR) approaches zero beyond 3X across all depths. For extra safety, a minimum depth of 5X was therefore chosen.