SSR genotyping protocol used for foreground selection during the NIL development process

For SSR genotyping, DNA was extracted from leaf samples collected from 2-week-old plants. Young leaf samples were collected and freeze-dried. Geno-grinder was used to grind these leaf samples and DNA was extracted using a modified CTAB method **(Murray and Thomson, 1980)** in deep-well plates. 0.8% agarose gel was used to check the quality and quantity of the extracted DNA and samples were then diluted to a final concentration of 20 ng μL^{-1} with TE (Tris-EDTA) buffer. The method described by **Panaud et al. (1996)** was used to perform polymerase chain reaction (PCR). Upon completion of PCR, 4 µL of 6X loading dye was added to the product and 4 µL of this solution mix was loaded into an 8% (w/v) polyacrylamide gel **(Sambrook et al., 1989)** for size separation of the amplified DNA fragments. The mini vertical electrophoresis system (CBS Scientific, model MGV-202–33) was used to conduct the electrophoresis. SYBR® Safe gel stain (Invitrogen) was used to stain the separated DNA fragments. The fragments were then visualized using a UV trans-illuminator.

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