# DNA preparation of Amorphophallus paeoniifolius for SSR evaluation

**Edi Santosa1, Chun Lan Lian2, Nobuo Sugiyama3, Raj Shekhar Misra4, Patchareeya Boonkorkaew5, Kanokwan Thanomchit5**

## Abstract

**Citation:** Edi Santosa1, Chun Lan Lian2, Nobuo Sugiyama3, Raj Shekhar Misra4, Patchareeya Boonkorkaew5, Kanokwan Thanomchit5 DNA preparation of Amorphophallus paeoniifolius for SSR evaluation. **protocols.io**
dx.doi.org/10.17504/protocols.io.ht3b6qn
**Published:** 24 Aug 2017

## Protocol

### Leaf collection

**Step 1.**

Leaf of Amorphophallus paeoniifolius was collected by using dry method. Fresh leaf at green stage was harvested. The leaf cleaned initially both abaxial and adaxial sides using moist tissue paper. Then, additional cleaning using mild detergent was required. The cleaning step was important to ensure extracted DNA only from the A. paeoniifolius. Then, the leaf midrib/vein was removed using sterile knife. Before stored in the silica gel, the leaf was cut into small sections facilitationg quick drying. One gram of fresh leaf required approximately 20-30 gram of silica gel, and stored in air tight box.

### DNA extraction

**Step 2.**

DNA was extracted using the modified CTAB method from the original protocol described by Lian et al. [2002]. Modification was required because the leaf of *A. paeoniifolius* contained glucomannan, a kind of high molecular of carbohydrate that difficult to dissolve in the water. Presence of glucomannan in the solution might cause DNA impurity, resulting on low PCR amplification. To separate DNA from the glucomannan, we used fishing method. Glucomannan pellet commontly transparent but still visible tobe observed at the bottom of the extraction tube after we added water. After about 40 minute, and DNA was expected dissolve competely, we pick up the glucomannan by fishing. For SSR analysis, DNA from approximately one gram of dry leaf was then preserved in a final volume of 200 µL of water and was stored at -30 °C until ready for use.

### SSR primer optimation and reagent

**Step 3.**

Polymerase chain reaction (PCR) was carried out in a 5-µL tube. For large sample, we recommend to use PCR plate with 48 wells. A 0.5-µL aliquot of extract was used in each PCR amplification to approximately contain 5-20 ng of DNA. SSR marker and DNA were amplified with a PCR reagent mix of 0.5 µM of the Forward primer, 0.1 µm of the Reverse primer tailed with U-19 (Fastac Co, Tokyo-

Japan), 0.5 µM of the U-19 primer labeled with Texas Red, 0.2 mM of each dNTP, 1× PCR buffer ($Mg^{2+}$ free, Applied Biosystems), 2.5 mM $MgCl_2$, and 0.5 U of Ampli Taq Gold DNA polymerase (Applied Biosystems). The PCR was performed using hot start to increase reproducibility of the product. The PCR was performed as follows: one cycle of 9 min at 94 °C followed by 30 s at the locus-specific annealing temperature ($T_a$) plus 1 min at 72 °C, and then 38 cycles of 30 s at 94 °C, 30 s at the locus-specific $T_a$ plus 1 min at 72 °C, followed by one cycle of 30 s at 94 °C, 30 s at the locus-specific $T_a$ plus 5 min extension at 72 °C, and ending at 4 °C. In the optimation step, we mixed some SSR primers that had similar *Ta* and the target bands that had distant allele size.

## Gel eletrophoresis

**Step 4.**

Finally, 1.5 µL of the PCR product was mixed with 3.5 µL of loading dye, denatured using the Thermal cycle TAKARA for five minutes at 95 °C, and then was placed on ice immediately for five minute prior to loading. Two-microliter aliquots were then loaded onto a 6% denaturing polyacrylamide gel and were run using the SQ-5500 sequencer (Hitachi Co., Tokyo). The band patterns were analyzed with FRAGYLS ver. 2 (Hitachi Electronics Engineering Co., Japan). Two bands were considered different alleles if the difference was larger than two base pairs.

Two bp different is a matter of technical procedure, not related to nucleotide repeat motif. Allele size was initially determined using FRAGYLS software. The software has automatically detected homo or hetero alleles, succesfully in most individuals. In some cases, hetero alleles had only one base in different of the first and the second alleles. In this case, we checked the size manually considering the bands quality, and concluded the alleles size as homo or hetero to avoid bias estimate of FRAGYLS software. Theoritically, we concluded the hetero alleles if 2 or more bp in different.