# The Appearance of DNA Bands Pattern Based on The Result of Primary Selection of RAPD Orchid Phaius spp.


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## INTRODUCTION

Phaius spphave flowers with large sizes and varied colors so that the demand and popularity of orchids increases and has the potential to become a promising business, especially as potted plants and cut flower stalks. The method used to increase genetic diversity and increase the population of orchids is by plant breeding. Genetic diversity is the basis of plant breeding, one of the main factors in genetic analysis is the selection of primers with the right DNA sequence for high amplification results with RAPD markers (Castro et al. 2012)

## METHODOLOGY

### DNA ISOLATION

One gram of young leaves was cleaned and placed in an Eppendorf tube with a capacity of 2 mL, with 5 mL of extraction buffer comprising 2 percent CTAB, 100 Mm Tris HCl pH 8, NaCl 1.4 M, 20 Mm EDTA, and 1 percent mercaptoethanol, plus 0.1g PVPP 40g and liquid nitrogen. The supernatant was separated by centrifugation at 11,000 rpm for 10 minutes at 4oC after it was incubated for 5 minutes at 65oC.DNA in the supernatant was purified by CIAA in a ratio of 24:1, centrifuged at 11,000 rpm for 10 minutes at 4OC. The DNA in the supernatant was purified with CIAA in a 24:1 ratio, centrifuged and transferred to a fresh tube, incubated for 30 minutes, then centrifuged for 10 minutes at 11,000 rpm. The liquid was thrown away, and the DNA was cleaned with 70% ethanol. The DNA precipitate was dissolved in 1 ml of TE buffer containing 1/10 of the total volume of 3M sodium acetate with a pH of 5.2. The liquid was frozen for 30 minutes before being centrifuged at 14,000 rpm for 10 minutes. The DNA palette has been cleaned and dried.[16]

### QUANTITY TEST OF DNA

Agarose gel was placed in a mold containing TAE buffer, the DNA sample was mixed with loading dye and then electrophoresed for 57 minutes at a voltage of 50 volts. The electrophoresis findings were examined using a UV transluminator before being photographed with a camera. The mixture was then dissolved in 500 mL of TE buffer and kept at 20°C. DNA amplification was performed for 3 hours using DNA, Primer mastermix (MgCl, tap polymerase, dNTPs, and water) and a PCR equipment.[16]

## RESULT

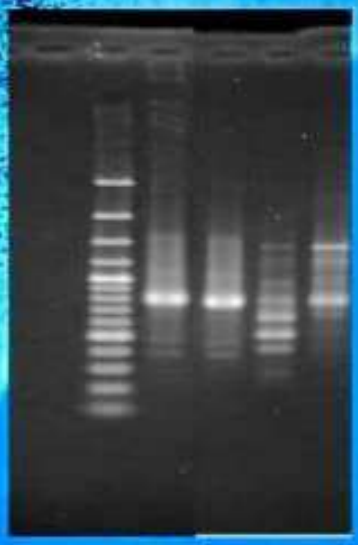

FIGURE 1.

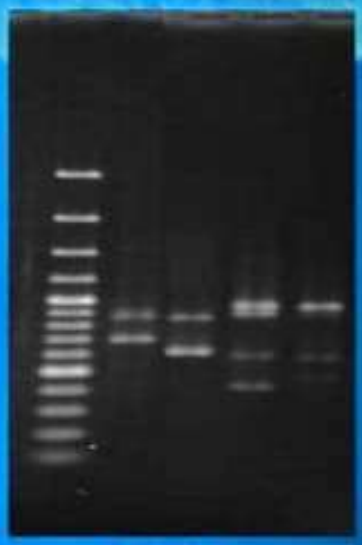

FIGURE 2.

Primers PCR results of Phaius tankervillae, Phaius montanus,Phaius collasus orchids , Phaius amboinensis with OPA 13 and OPB 18 primers produced 6 and 4 DNA bands, respectively, with an average of 6 DNA bands per primer. Band resolution is not clearly
visible, this is due to differences in amplified DNA fragments. The more amplified DNA fragments, the stronger the DNA band resolution. The same band pattern indicates closeness or kinship. Species of Phaius spp that have close genetic diversity, namely Phaius tankervillae, and Phaius amboinensis. (Figures 1 and 2).

## CONCLUSION

2 primers RAPD OPA 13 and OPB 18 can be used for DNA amplification oforchids Phaius spp (Phaius tankervillae, Phaius montanus Phaius collasus and Phaius amboinensis) because they produce clear DNA bands, these primers can amplify as many as 25 DNA bands with 100% polymorphism . The Phaius tankervillae and Phaius amboinensis orchids have close genetic diversity, characterized by the arrangement of bands located in the same base arrangement.