# APPENDICES

# APPENDIX A

## Chemical reagents preparation

**CTAB buffer**

| | |
|---|---|
| 5M NaCl | 28 ml |
| 1M Tris-HCl pH 8 | 10 ml |
| 0.5 mM EDTA pH8 | 4 ml |
| CTAB | 2 g |
| PVPP | 1 g |
| Add $H_2O$ to | 100 ml |

**SDS buffer**

| | |
|---|---|
| 2x Extraction buffer stock | |
| 0.6M NaCl | 6 ml |
| 1M Tris-HCl pH 7.5 | 5 ml |
| 40 mM EDTA pH8 | 16 ml |
| SDS | 0.5 g |
| Urea | 30 g |
| 10 mM 2β-mercaptoethanol | 7.8 µl |
| Phenol | 5% v/v |
| Add $H_2O$ to | 50 ml |

**Wash buffer**

| | |
|---|---|
| Ammonium acetate | 10 mM |
| Ethanol | 75% |

**TE buffer**

| | |
|---|---|
| 2M Tris-HCl pH 8 | 500 µl |
| 0.5 mM EDTA pH8 | 200 µl |
| Added dd$H_2O$ to | 500 ml |

**0.5M EDTA pH 7.5 and 8**

EDTA ($Na_2.2H_2O$) 186.1 g

Added dd$H_2O$ to 1000 ml

Adjust pH as needed

**1.5% Agarose gel**

Agarose 1.5 g

1X TBE 100 ml

# APPENDIX B

**Appendix table 1 Nucleotide sequence of RAPD primer**

| Primer | | Primer | |
|---|---|---|---|
| Primer | Sequences 5’-3’ | Primer | Sequences 5’-3’ |
| OPA-01 | 5’-CAG GCC CTT C-3’ | OPA-11 | 5’-CAA TCG CCG T-3’ |
| OPA-02 | 5’-TGC CGA GCT G-3’ | OPA-12 | 5’-TCG GCG ATA G-3’ |
| OPA-03 | 5’-AGT CAG CCA C-3’ | OPA-13 | 5’-CAG CAC CCA C-3’ |
| OPA-04 | 5’-AAT CGG GCT G-3’ | OPA-14 | 5’-TCT GTG CTG G-3’ |
| OPA-05 | 5’-AGG GGT CTT G-3’ | OPA-15 | 5’-TTC CGA ACC C-3’ |
| OPA-06 | 5’-GGT CCC TGA C-3’ | OPA-16 | 5’-AGC CAG CGA A-3’ |
| OPA-07 | 5’-GAA ACG GGT G-3’ | OPA-17 | 5’-GAC CGC TTG T-3’ |
| OPA-08 | 5’-GTG ACG TAG G-3’ | OPA-18 | 5’-AGG TGA CCG T-3’ |
| OPA-09 | 5’-GGG TAA CGC C-3’ | OPA-19 | 5’-CAA ACG TCG G-3 |
| OPA-10 | 5’-GTG ATC GCA G-3’ | OPA-20 | 5’-GTT GCG ATC C-3’ |
| | | | |
| OPC-01 | 5’-TTC GAG CCA G-3’ | OPC-11 | 5’-AAA GCT GCG G-3’ |
| OPC-02 | 5’-GTG AGG CGT C-3’ | OPC-12 | 5’-TGT CAT CCC C-3’ |
| OPC-03 | 5’-GGG GGT CTT T-3’ | OPC-13 | 5’-AAG CCT CGT C-3’ |
| OPC-04 | 5’-CCG CAT CTA C-3’ | OPC-14 | 5’-TGC GTG CTT G-3’ |
| OPC-05 | 5’-GAT GAC CGC C-3’ | OPC-15 | 5’-GAC GGA TCA G-3’ |
| OPC-06 | 5’-GAA CGG ACT C-3’ | OPC-16 | 5’-CAC ACT CCA G-3’ |
| OPC-07 | 5’-GTC CCG ACG A-3’ | OPC-17 | 5’-TTC CCC CCA G-3’ |
| OPC-08 | 5’-TGG ACC GGT G-3’ | OPC-18 | 5’-TGA GTG GGT G-3’ |
| OPC-09 | 5’-CTC ACC GTC C-3’ | OPC-19 | 5’-GTT GCC AGC C-3’ |
| OPC-10 | 5’-TGT CTG GGT G-3’ | OPC-20 | 5’-ACT TCG CCA C-3’ |
| | | | |
| OPD-01 | 5’-ACC GCG AAG G-3’ | OPD-11 | 5’-AGC GCC ATT G-3’ |
| OPD-02 | 5’-GGA CCC AAC C-3’ | OPD-12 | 5’-CAC CGT ATC C-3’ |
| OPD-03 | 5’-GTC GCC GTC A-3’ | OPD-13 | 5’-GGG GTG ACG A-3’ |

| Primer | Sequences 5’-3’ | Primer | Sequences 5’-3’ |
|---|---|---|---|
| OPD-04 | 5’-TCT GGT GAG G-3’ | OPD-14 | 5’-CTT CCC CAA G-3’ |
| OPD-05 | 5’-TGA GCG GAC A-3’ | OPD-15 | 5’-CAT CCG TGC T-3’ |
| OPD-06 | 5’-ACC TGA ACG G-3’ | OPD-16 | 5’-AGG GCG TAA G-3’ |
| OPD-07 | 5’-TTG GCA CGG G-3’ | OPD-17 | 5’-TTT CCC ACG G-3’ |
| OPD-08 | 5’-GTG TGC CCC A-3’ | OPD-18 | 5’-GAG AGC CAA C-3’ |
| OPD-09 | 5’-CTC TGG AGA C-3’ | OPD-19 | 5’-CTG GGG ACT T-3’ |
| OPD-10 | 5’-GGT CTA CAC C-3’ | OPD-20 | 5’-ACC CGG TCA C-3’ |
| | | | |
| OPF-01 | 5’-ACG GAT CCT G-3’ | OPF-11 | 5’-TTG GAT CCC C-3’ |
| OPF-02 | 5’-CCT GAT CAC C-3’ | OPF-12 | 5’-ACG GTA CCA G-3’ |
| OPF-03 | 5’-CCG AAT TCC C-3’ | OPF-13 | 5’-GGC TGC AGA A-3’ |
| OPF-04 | 5’-GGT GAT CAG G-3’ | OPF-14 | 5’-TGC TGC AGG T-3’ |
| OPF-05 | 5’-CCG AAT TCC C-3’ | OPF-15 | 5’-CCA GTA CTC C-3’ |
| OPF-06 | 5’-GGG AAT TCG G-3’ | OPF-16 | 5’-GGA GTA CTG G-3’ |
| OPF-07 | 5’-CCG ATA TCC C-3’ | OPF-17 | 5’-AAC CCC GGA A-3’ |
| OPF-08 | 5’-GGG ATA TCG G-3’ | OPF-18 | 5’-TTC CCG GGT T-3’ |
| OPF-09 | 5’-CCA AGC TTC C-3’ | OPF-19 | 5’-CCT CTA GAC C-3’ |
| OPF-10 | 5’-GGA AGC TTG G-3’ | OPF-20 | 5’-GGT CTA GAG G-3’ |
| | | | |
| OPG-01 | 5’-CTACGGAGGA-3’ | OPG-11 | 5’-TGC CCG TCG T-3’ |
| OPG-02 | 5’-GGC ACT GAG G-3’ | OPG-12 | 5’-CAG CTC ACG A-3’ |
| OPG-03 | 5’-GAG CCC TCC A-3’ | OPG-13 | 5’-CTC TCC GCC A-3’ |
| OPG-04 | 5’-AGC GTG TCT G-3’ | OPG-14 | 5’-GGA TGA GAC C-3’ |
| OPG-05 | 5’-CTG AGA CGG A-3’ | OPG-15 | 5’-ACT GGG ACT C-3’ |
| OPG-06 | 5’-GTG CCT AAC C-3’ | OPG-16 | 5’-AGCGTC CTC C-3’ |
| OPG-07 | 5’-GAA CCT GCG G-3’ | OPG-17 | 5’-ACG ACC GAC A-3’ |
| OPG-08 | 5’-TCA CGT CCA C-3’ | OPG-18 | 5’-GGC TCA TGT G-3’ |
| OPG-09 | 5’-CTG ACG TCA C-3’ | OPG-19 | 5’-GTC AGG GCA A-3’ |
| OPG-10 | 5’-AGG GCC GTC T-3’ | OPG-20 | 5’-TCT CCC TCA G-3’ |

| Primer | Sequences 5’-3’ | Primer | Sequences 5’-3’ |
|---|---|---|---|
| OPN-01 | 5’-CTC ACG TTG G-3’ | OPN-11 | 5’-TCG CCG CAA A-3’ |
| OPN-02 | 5’-ACC AGG GGC A-3’ | OPN-12 | 5’-CAC AGA CAC C-3’ |
| OPN-03 | 5’-GGT ACT CCC C-3’ | OPN-13 | 5’-AGC GTC ACT C-3’ |
| OPN-04 | 5’-GAC CGA CCC A-3’ | OPN-14 | 5’-TCG TGC GGG T-3’ |
| OPN-05 | 5’-ACT GAA CGC C-3’ | OPN-15 | 5’-CAG CGA CTG T-3’ |
| OPN-06 | 5’-GAG ACG CAC A-3’ | OPN-16 | 5’-AAG CGA CCT G-3’ |
| OPN-07 | 5’-CAG CCC AGA G-3’ | OPN-17 | 5’-CAT TGG GGA G-3’ |
| OPN-08 | 5’-ACC TCA GCT C-3’ | OPN-18 | 5’-GGT GAG GTC A-3’ |
| OPN-09 | 5’-TGC CGG CTT G-3’ | OPN-19 | 5’-GTC CGT ACT G-3’ |
| OPN-10 | 5’-ACAACT GGG G-3’ | OPN-20 | 5’-GGT GCT CCG T-3’ |
| | | | |
| OPU-01 | 5’-ACGGACGTCA-3’ | OPU-11 | 5’-AGACCCAGAG-3’ |
| OPU-02 | 5’-CTGAGGTCTC-3’ | OPU-12 | 5’-TCACCAGCCA-3’ |
| OPU-03 | 5’-CTATGCCGAC-3’ | OPU-13 | 5’-GGCTGGTTCC-3’ |
| OPU-04 | 5’-ACCTTCGGAC-3’ | OPU-14 | 5’-TGGGTCCCTC-3’ |
| OPU-05 | 5’-TTGGCGGCCT-3’ | OPU-15 | 5’-ACGGGCCAGT-3’ |
| OPU-06 | 5’-ACCTTTGCGG-3’ | OPU-16 | 5’-CTGCGCTGGA-3’ |
| OPU-07 | 5’-CCTGCTCATC-3’ | OPU-17 | 5’-ACCTGGGGAG-3’ |
| OPU-08 | 5’-GGCGAAGGTT-3’ | OPU-18 | 5’-GAGGTCCACA-3’ |
| OPU-09 | 5’-CCACATCGGT-3’ | OPU-19 | 5’-GTCAGTGCGG-3’ |
| OPU-10 | 5’-ACCTCGGCAC-3’ | OPU-20 | 5’-ACAGCCCCCA-3’ |

# APPENDIX C

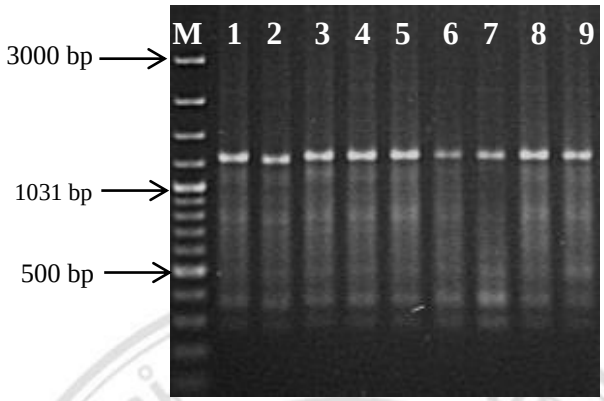


Figure 1 RAPD profile of *Habenaria* and *Pecteilis* generated by OPA01 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*]

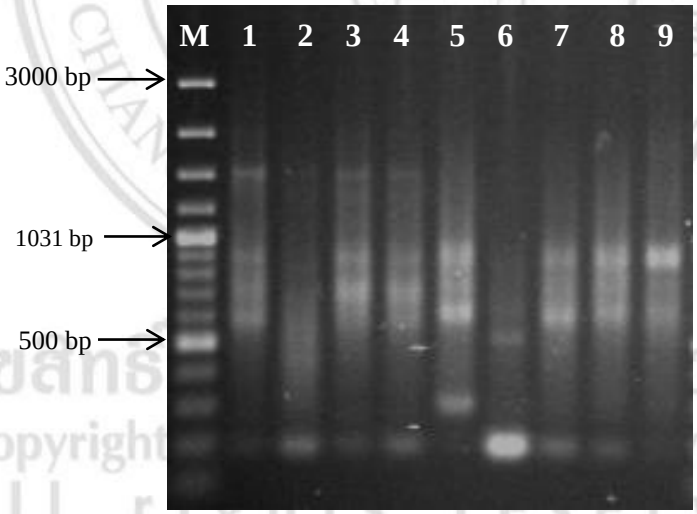


Figure 2 RAPD profile of *Habenaria* and *Pecteilis* generated by OPA03 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*]

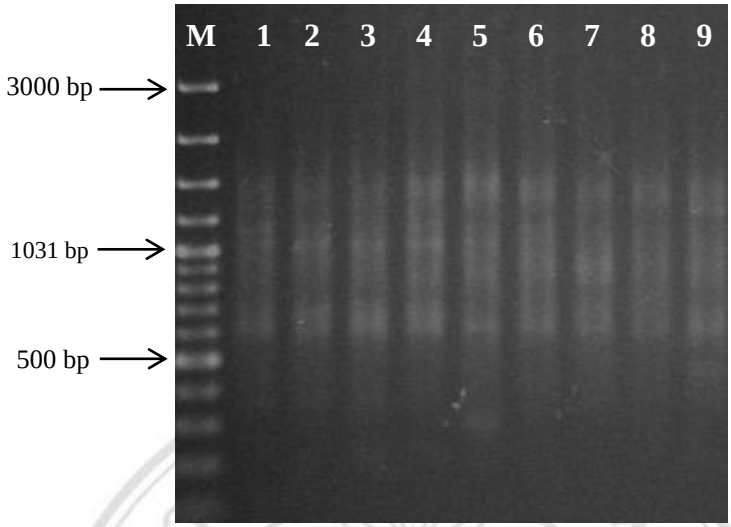


Figure 3 RAPD profile of *Habenaria* and *Pecteilis* generated by OPA13 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*]

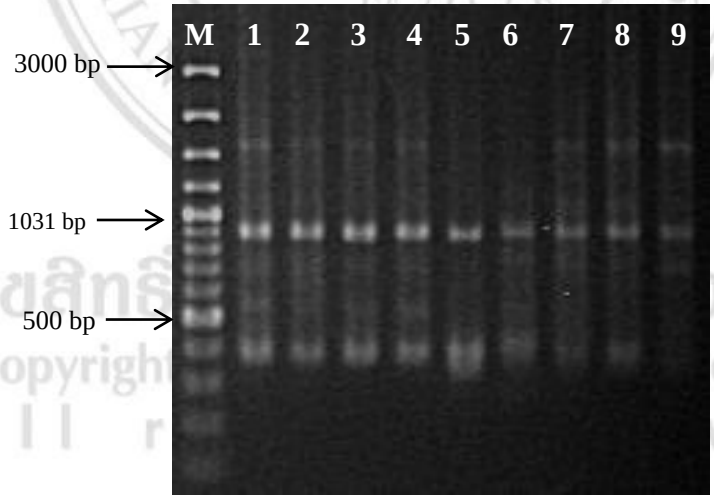


Figure 4 RAPD profile of *Habenaria* and *Pecteilis* generated by OPC02 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*]

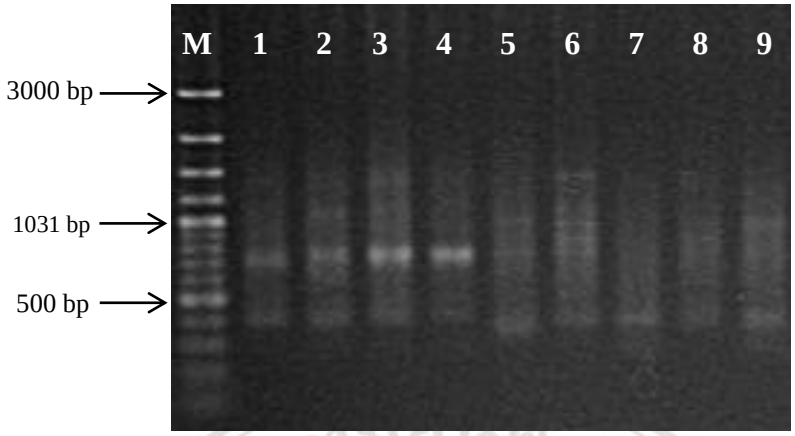


Figure 5 RAPD profile of *Habenaria* and *Pecteilis* generated by OPC20 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*]

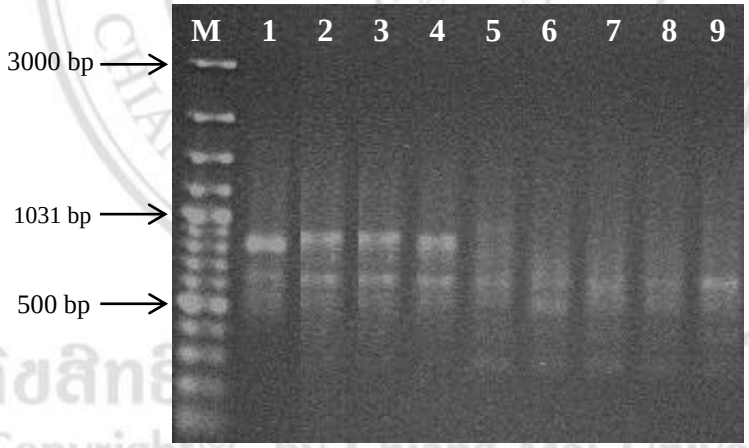


Figure 6 RAPD profile of *Habenaria* and *Pecteilis* generated by OPD02 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*]

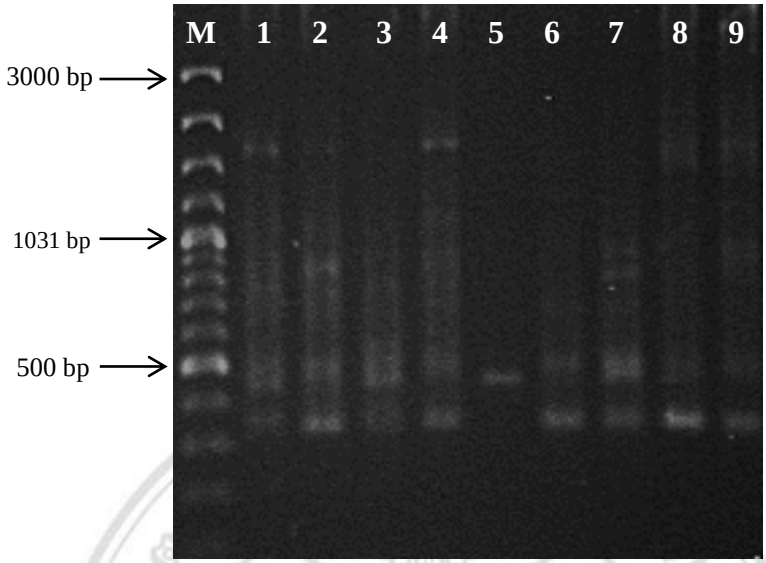


Figure 7 RAPD profile of *Habenaria* and *Pecteilis* generated by OPD03 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*]

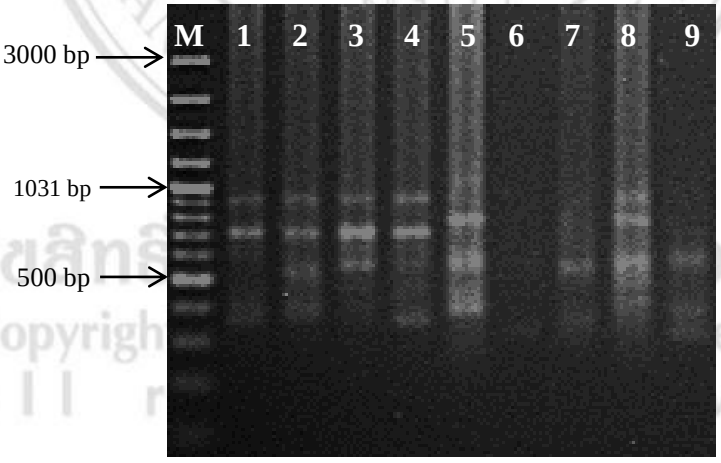


Figure 8 RAPD profile of *Habenaria* and *Pecteilis* generated by OPD11 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*]

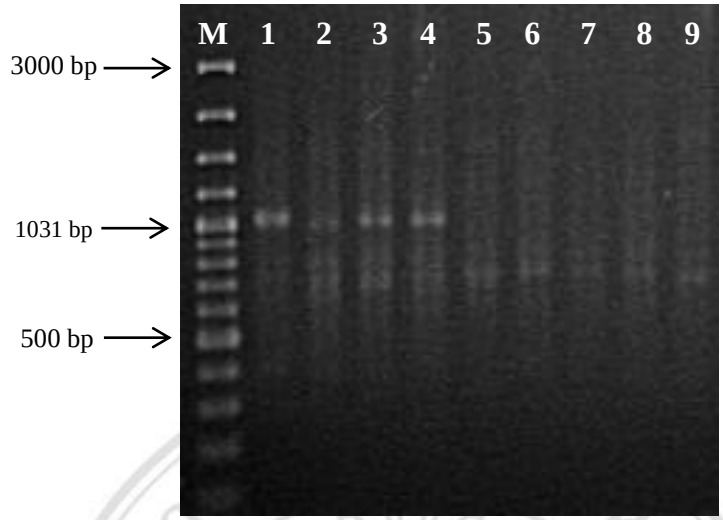


Figure 9 RAPD profile of *Habenaria* and *Pecteilis* generated by OPF02 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*]

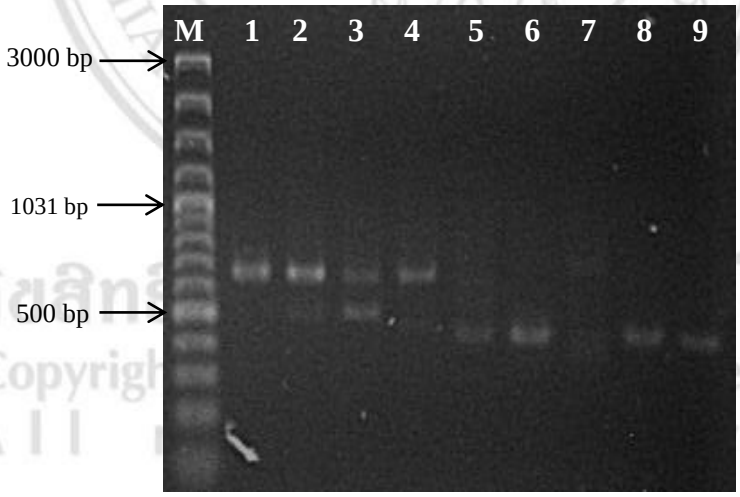


Figure 10 RAPD profile of *Habenaria* and *Pecteilis* generated by OPF08 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*]

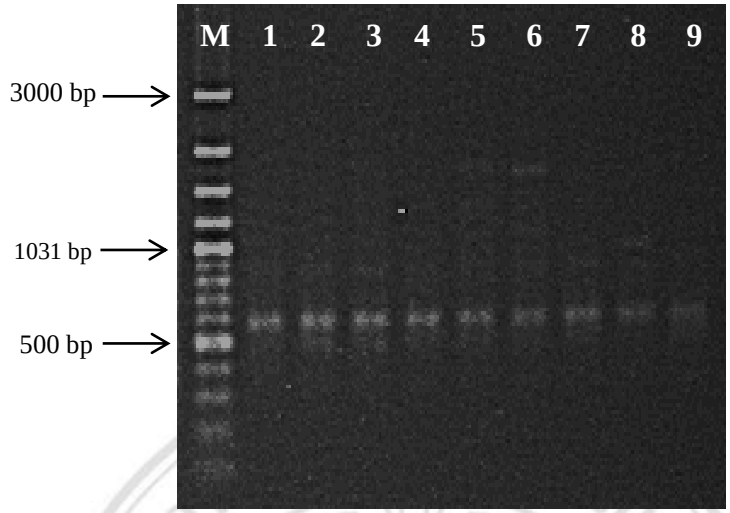


Figure 11 RAPD profile of *Habenaria* and *Pecteilis* generated by OPF10 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*]

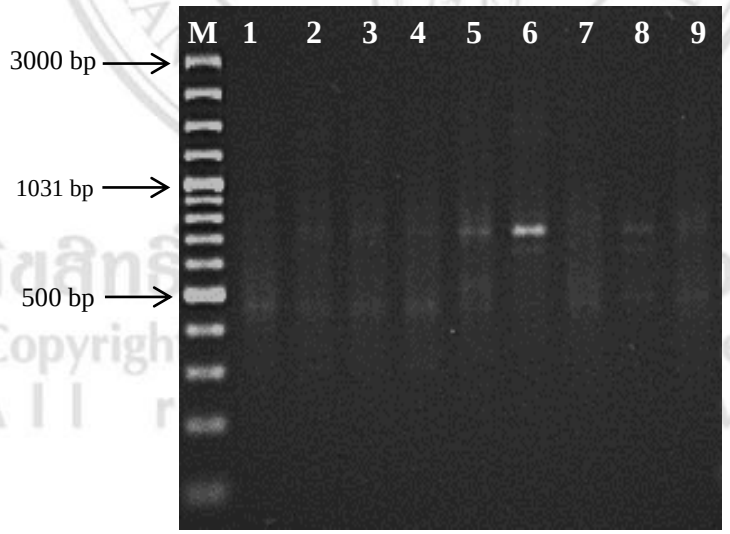


Figure 12 RAPD profile of *Habenaria* and *Pecteilis* generated by OPF13 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*]

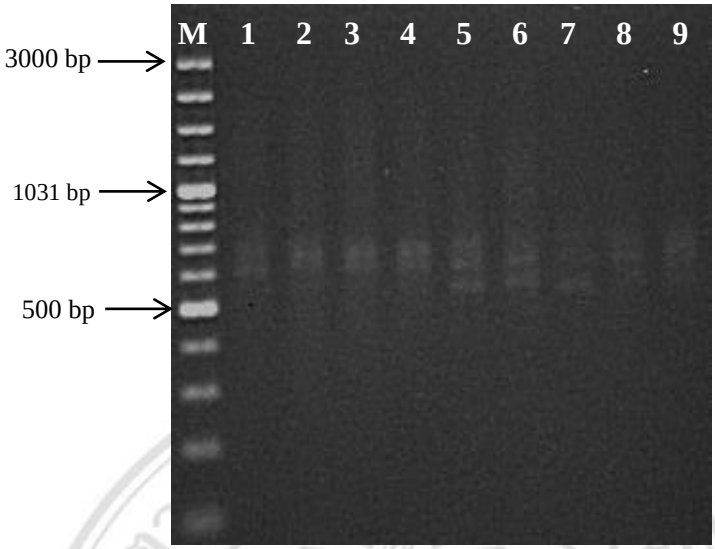


Figure 13 RAPD profile of *Habenaria* and *Pecteilis* generated by OPF16 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*]

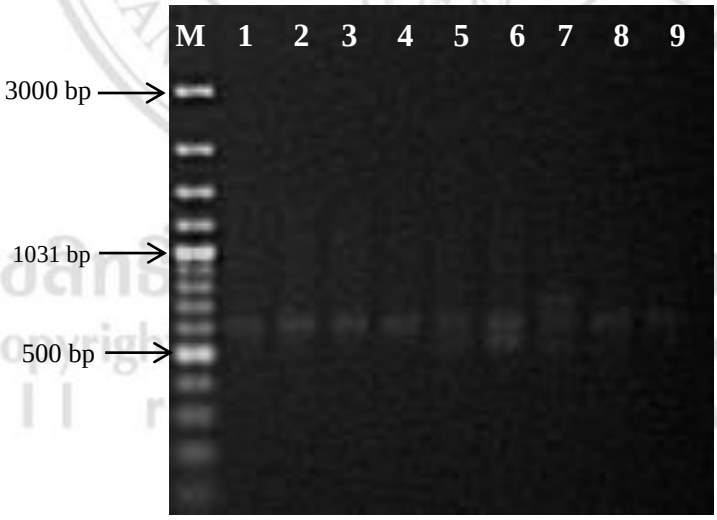


Figure 14 RAPD profile of *Habenaria* and *Pecteilis* generated by OPF17 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*]

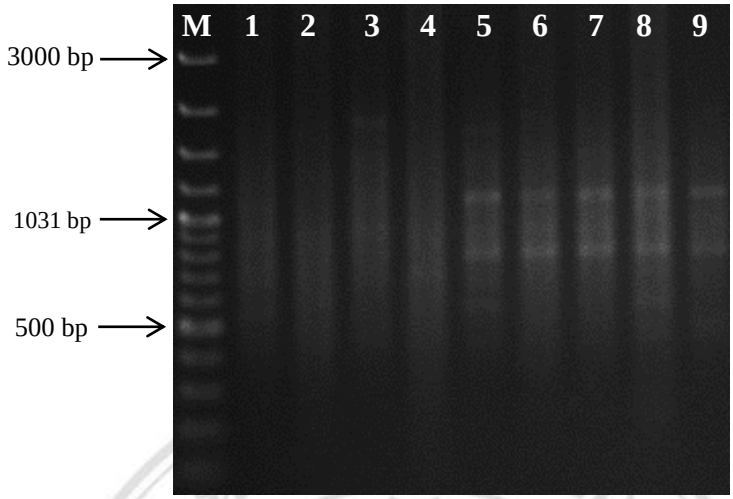


Figure 15 RAPD profile of *Habenaria* and *Pecteilis* generated by OPG01 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*]

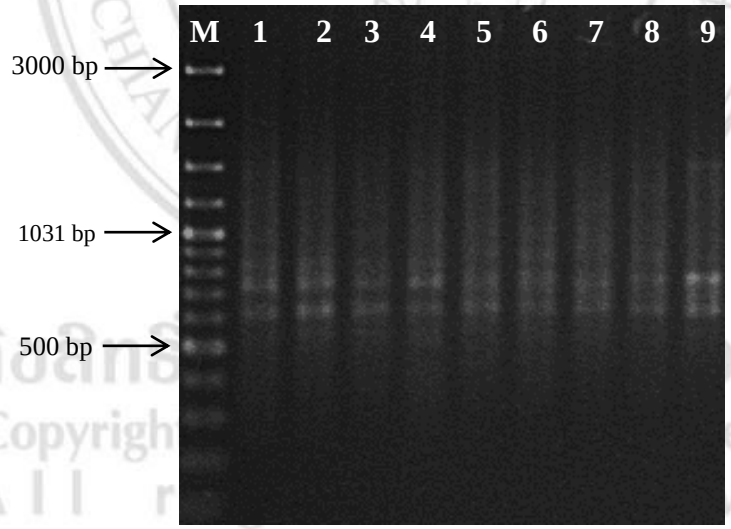


Figure 16 RAPD profile of *Habenaria* and *Pecteilis* generated by OPG05 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*]

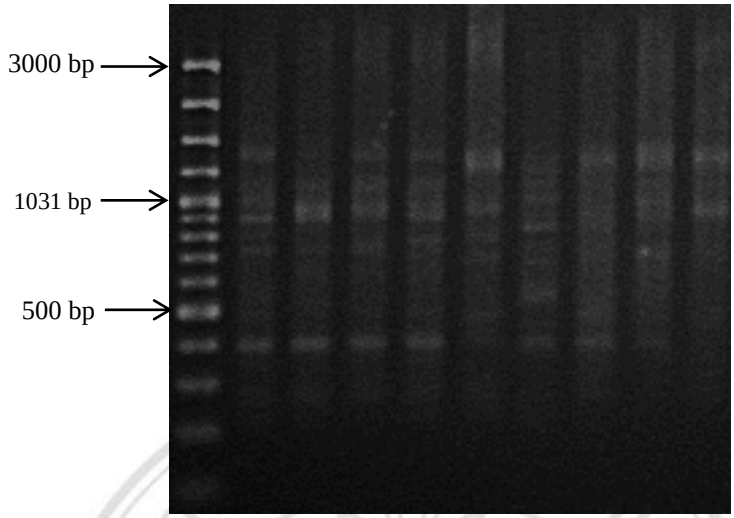


Figure 17 RAPD profile of *Habenaria* and *Pecteilis* generated by OPN07 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*]

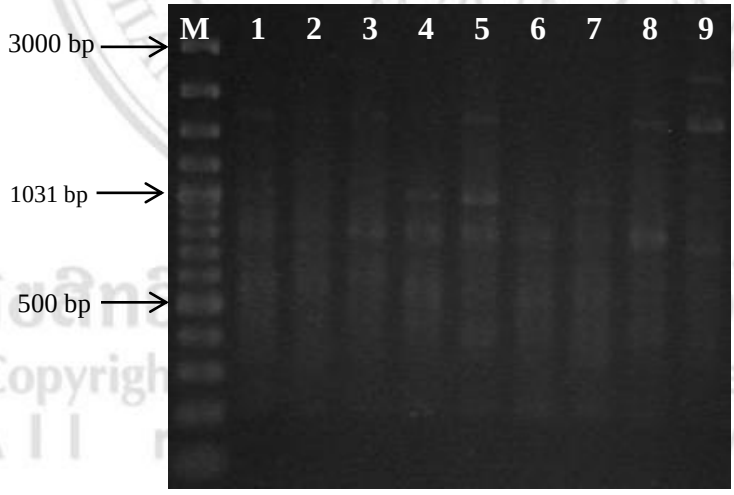


Figure 18 RAPD profile of *Habenaria* and *Pecteilis* generated by OPN13 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*]

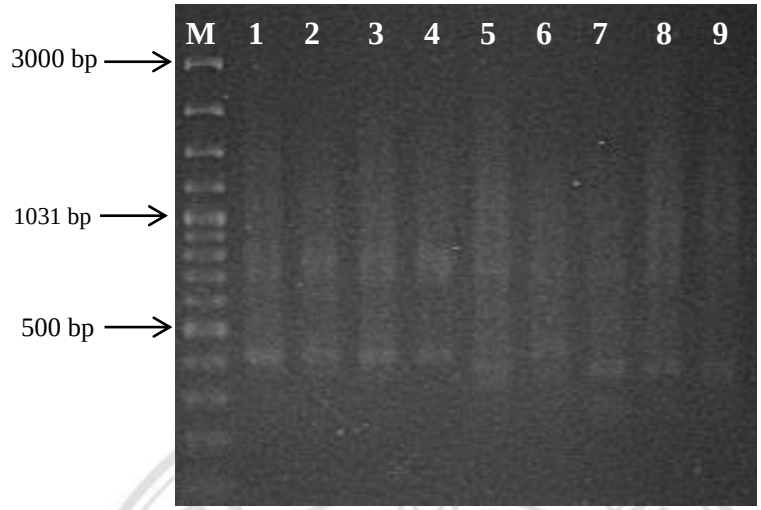


Figure 19 RAPD profile of *Habenaria* and *Pecteilis* generated by OPN14 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*]

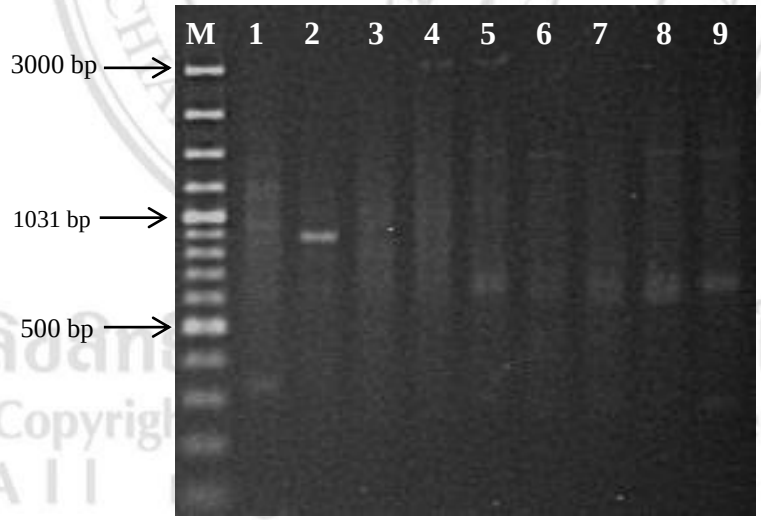


Figure 20 RAPD profile of *Habenaria* and *Pecteilis* generated by OPN17 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*]

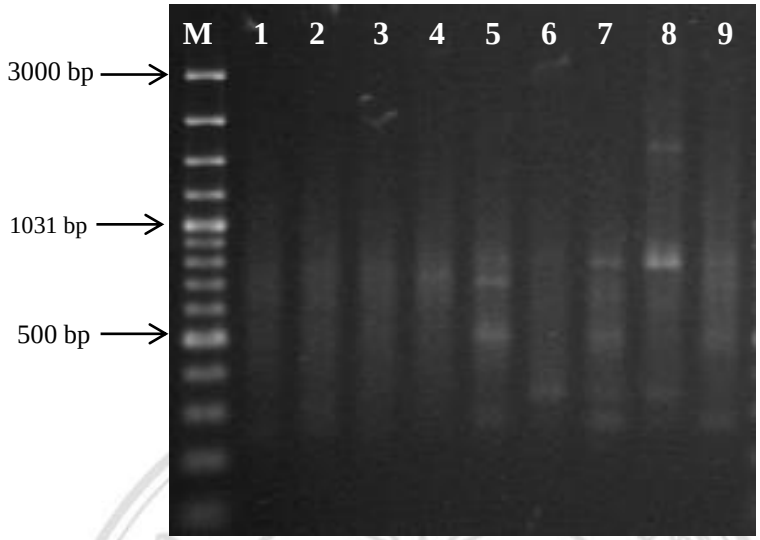


Figure 21 RAPD profile of *Habenaria* and *Pecteilis* generated by OPN18 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*]

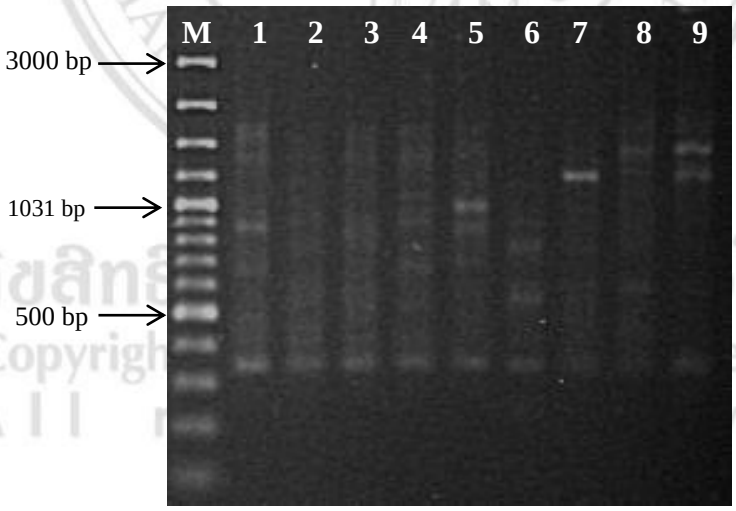


Figure 22 RAPD profile of *Habenaria* and *Pecteilis* generated by OPN19 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*]

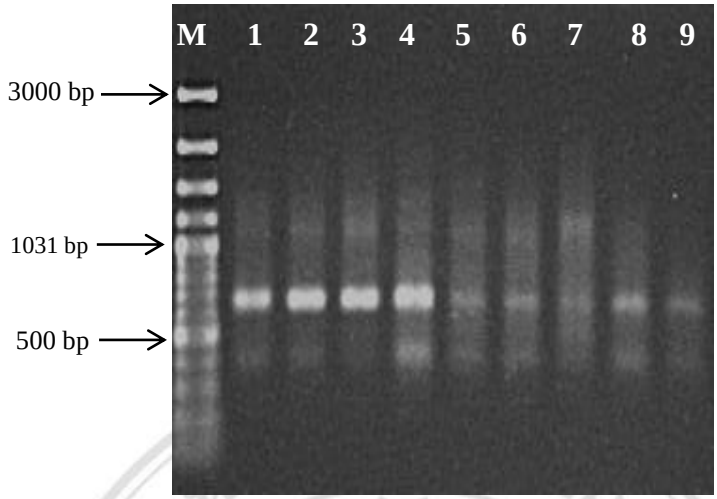


Figure 23 RAPD profile of *Habenaria* and *Pecteilis* generated by OPU02 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*]

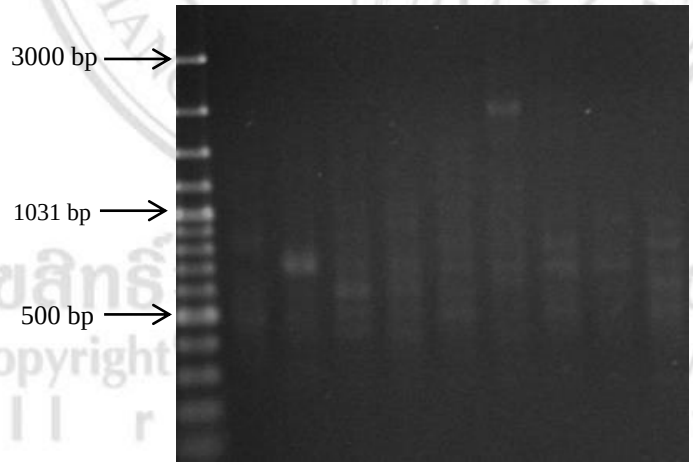


Figure 24 RAPD profile of *Habenaria* and *Pecteilis* generated by OPU03 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*]

# CURRICULUM VITAE

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