APPENDIX

Appendix 1 Wild rice survey sheet

Surveyor …….....
Date of survey…..……

1. Location no……Local name of wild rice……..…
2. Lat/Lon or UTM……
3. Address……..……city……province……..…….
4. Map

5. Habitat
   O In side rice field O Edge rice field O By road side O Abandoned
6. Area……..m^2/km^2 O patchy O all covered
7. Awn color O white O red
8. Awn length O 0-5 cm. O 5-10 cm. O >10 cm.
9. Growth stage O anthesis O milking stage O grain filling O seed maturity
10. Plant type O erect O prostrate
11. Stigma color O white O dark purple O other……
12. Anther length O 1/3 of spikelet O 1/2 of spikelet O same of spikelet
13. Type O annual O perennial O annual-perennial intermediate
14. Hull color O black O black with stripe O straw O other……
15. Special traits…….

Appendix 2 Descriptor state and Meaning of morphological and other characters of common wild and cultivated rice

Character No. and Name	Descriptor state and Meaning
1. G.S. No.	
2. Species name :	
3. Designation :	
4. Location :	
5. Growth habit :	1= erect; 3= semi-erect; 5= intermediate; 7= decumbent
6. Leaf sheath color :	1= green; 2= dark green; 3= light purple; 4= purple
7. Leaf blade color :	1= green; 2= dark green; 3= purple at margin; 4= purple line; 5= all purple
8. Tillers:	number of tiller per plant at full vegetative stage
9. Ligule color :	1= colorless 2= purple
10. Ligule shape :	1= 1clip 2= 2 clips 3= truncate
11. Auricle color :	1= colorless 2= purple
12. Internode color :	1= green 2= light green 3= purple line 4= purple
13. Flowering date (50%) or Maturity day	
14. Spikelet color :	1= straw 2= brown 3= yellow 4= red 5= black
15. Apiculus color :	1= colorless 2= brown 3= yellow 4= red 5= black
16. Anther length :	1=¼ spikelet 2= ½ spikelet 3= ¾ spikelet 4= full or equal to spikelet.
17. Stigma color :	1= colorless 2= red 3= purple 4= black
18. Stigma exsertion :	1= enclosed 2= red 3= well exserted
19. Panicle type :	1= compact 2= intermediate 3= open
20. Awn color :	0= no awn 2= white 3= red
21. Awn length :	0= no awn 1= 0-5cm 2= 5-10cm 3= >10cm
22. Pericarp color :	1= white 2= red

Appendix 3 DNA extraction

1. Extraction buffer:

DNA extraction	Stock	10 ml
4% CTAB	100%	0.4g
100 mM Tris – Hcl pH 8.0	1M	1ml
20 mM EDTA pH 8.0	0.5 M	0.4ml
1.4 M NaCl	5 M	2.8 ml
0.4% β-mercapto-ethanol	100%	40 µl
H_2O	100%	6ml

2. Extraction buffer 1ml into 0.1g of grinded leaf sample.

3. Vortex mixed ~ 15 – 30 (second) each tube.

4. Incubate 3 hours in 65°C of water bath and inverting every 30 min.

5. Centrifuge 5 min at 12000 rpm.

6. Supernatant transferring to the new tube and put Chlololform-isoamyl alcohol (24:1) (500µl). Gently inverting about 5min, and centrifuge at 12000 rpm for 20min.

7. Carefully transfer 500µl of the top aqueous phase (3 layer) into the new tube and then add (100 µl) with Sodium-acetate (3M NaoAC).

8. Add 600µl ice-cold Isopropanal Alcohol (-20°C) into the Supernatant and inverting every tube until homogeneous together.

9. Keep in the freezer (-20°C) overnight or (-70°C) at least 20 minutes.

10. Centrifuge for 10 minutes at (12,000 rpm).

11. Carefully discard supernatant. Keep only DNA in the bottom tube.

12. Add 500µl of 70% Ethanol (-20°C) for pellet rinsing.

13. Centrifuge for 5min. (12,000rpm).

14. Discard supernatant.

15. Add 500µl of 95% Ethanol (-20°C) for pellet rinsing.

16. Centrifuge for 5min. (12,000rpm).

17. Discard supernatant.

18. Vacuum dry for 15- 30 minutes.

19. Add 100 µl of 1x TE buffer and leave at room temperature for 10 minutes.

20. Store DNA samples at -20°C freezer.

Appendix 4 PCR Processing

1. Ice preparing in the box

2. Tube preparing (clean your hand with alcohol).

3. Make a cocktail for 20µl PCR per reaction.

PCR extraction	1 Reaction	25 Reactions
H_2O	15.34µl	383.5µl
10 X Buffer	2µl	50µl
50 mM MgCL	1µl	25µl
Primer (F)	0.2µl	5µl
Primer (R)	0.2µl	5µl
25 mM dNTP	0.16µl	4µl
Taq DNA polymerase	0.1µl	2.5µl

4. Cocktail tube vortex mix ~ 5 – 10 (second)

5. Take 19µl from cocktail tube to PCR tube.

6. Take 1µl from DNA tube (concentrate) to every reaction tube.

7. Put in PCR processing machine until machine shown forever and keep in freezer (-20°C).

Appendix 5 Polyacrylamine Gel Electrophoresis Preparing (PAGE)

1. Glass rinsing with (75% acohol).

2. Electrophoresis installing.

3. Take agarose 1% or 1g mixed with 1xTBE (100ml) into microwave until homogeneous and then put it on the bottom layer of electrophoresis (2-3ml).

4. 10% of polyacrylamine gel in lower layer:

Raw material	One gel
Water	15ml
5xTBE	6.5ml
Acrylamide: Bis (29:1)	11ml
APS	224µl
TEMED	11.5µl
Total	**32.5ml**

5. 4% of acrylamine gel in upper layer:

Raw material	One gel
Water	4.3ml
5xTBE	0.6ml
Mixed from lower	2ml
kept in freezer (4°C) until use	
APS	35µl
TEMED	4µl

6. Mixed lower together and take it into electrophoresis and quickly put ~ 2ml of Buthanol into upper of electrophoresis glass.

7. Waiting about 30 min or until the gel were polymerized.

8. Buthanol water pouring out from electrophoresis and than it's cleaning with the water.

9. Comb molding.

10. Put the upper concentration on the lower layer and waiting may be 30 min until to become coagulated again.

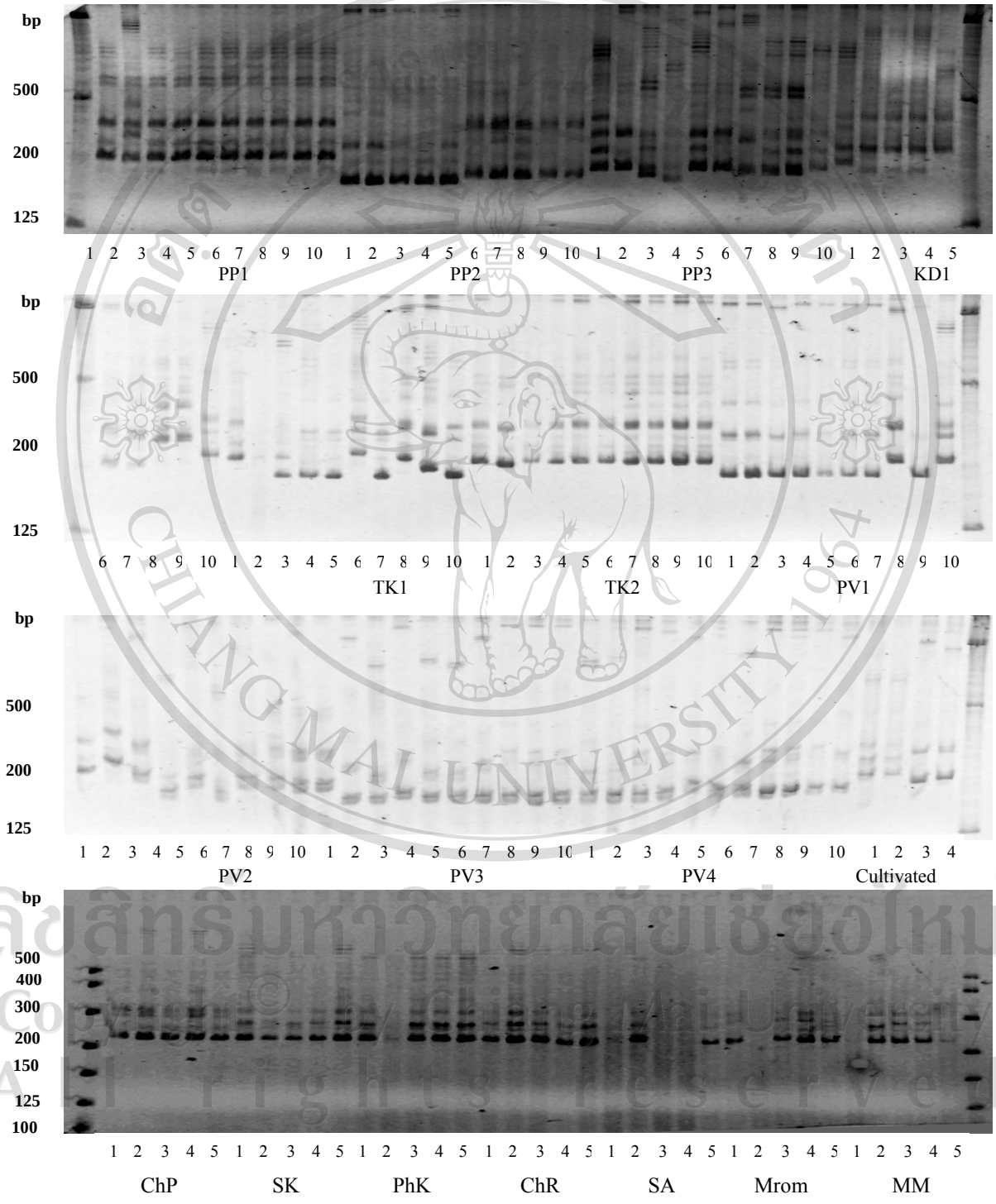
11. To prepare box electrophoresis with water (1xTBE).

12. Carefully to take the electrophoresis glass into a box electrophoresis.

13. Comb drawing out.

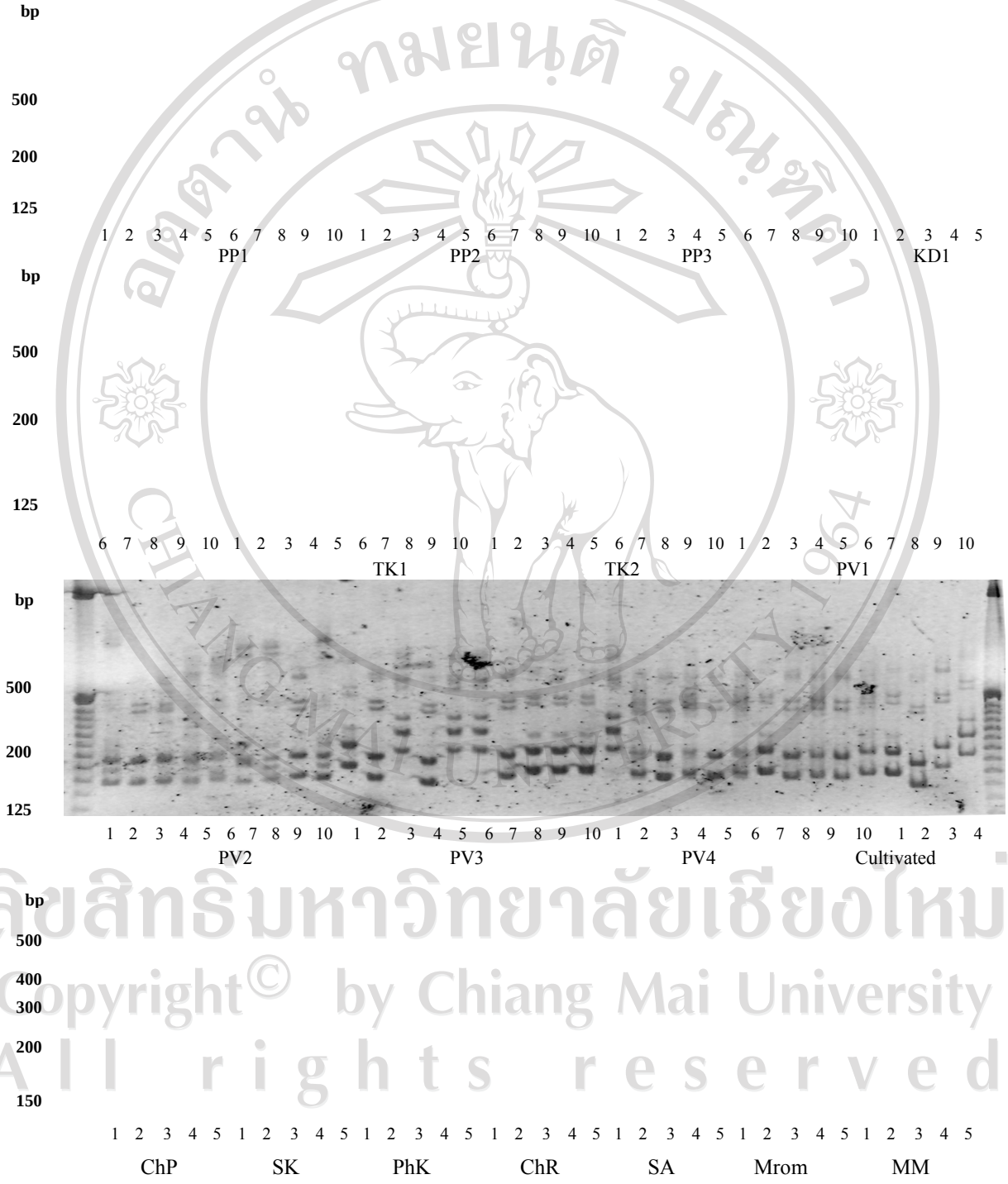
14. PCR (freezer) mixed loding dye (12-13µl) per each PCR tube and put in the hole.

Appendix 6 Band patterns of 10 wild rice populations and 7 local rices at RM20 lucus.



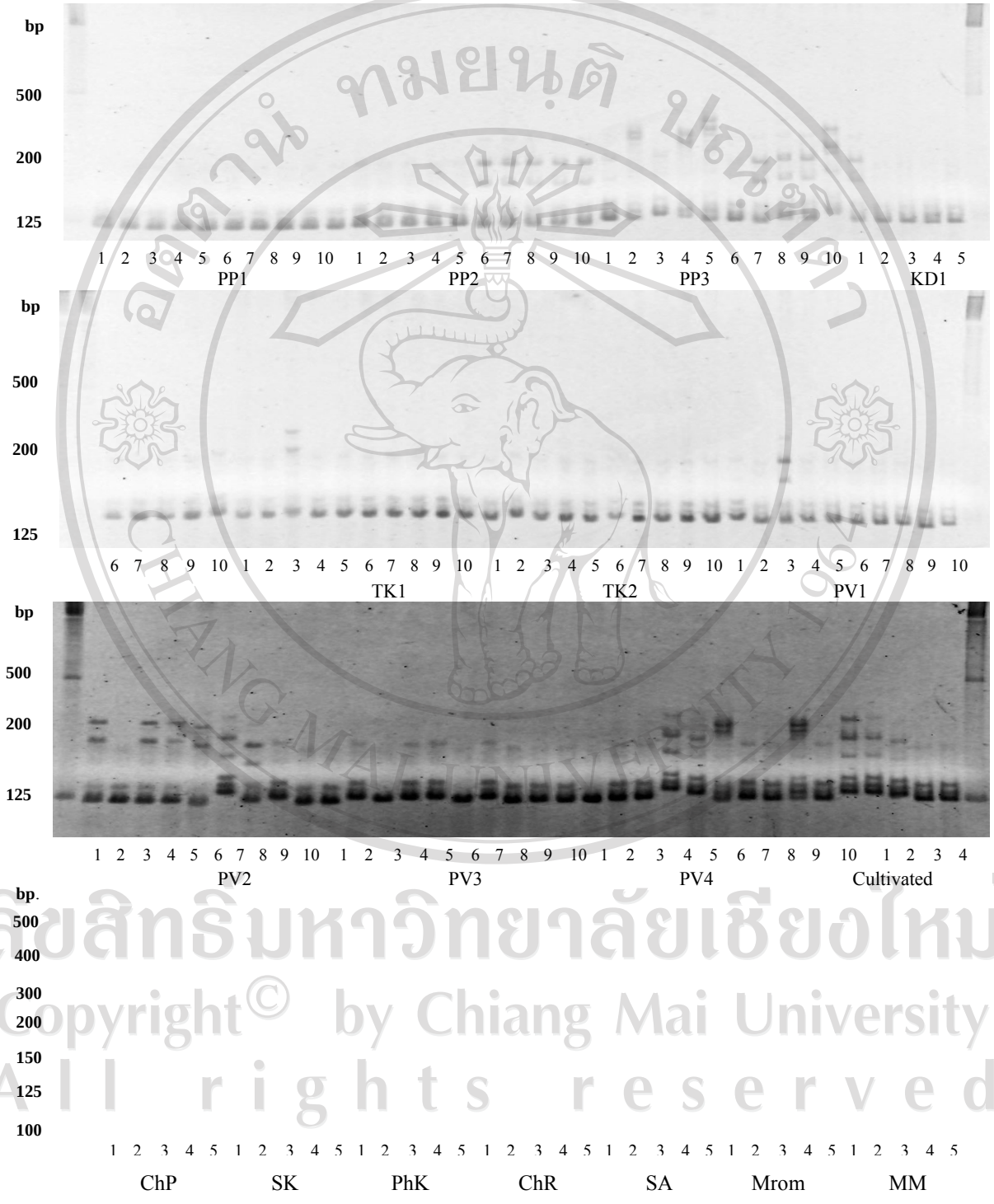
PP = Phnom Penh, KD = Kandal, TK = Takeo, PV = Prey Veng, ChP = Chhmar Prom, SK = Srau Krahorm, PhK = Phkar Khgney, ChR = Chomkoum Rumpak, SA = Sombok Angkrorng, MM = Mong Mang.

Appendix 7 Band patterns of 10 wild rice populations and 7 local rices at RM164 lucus.



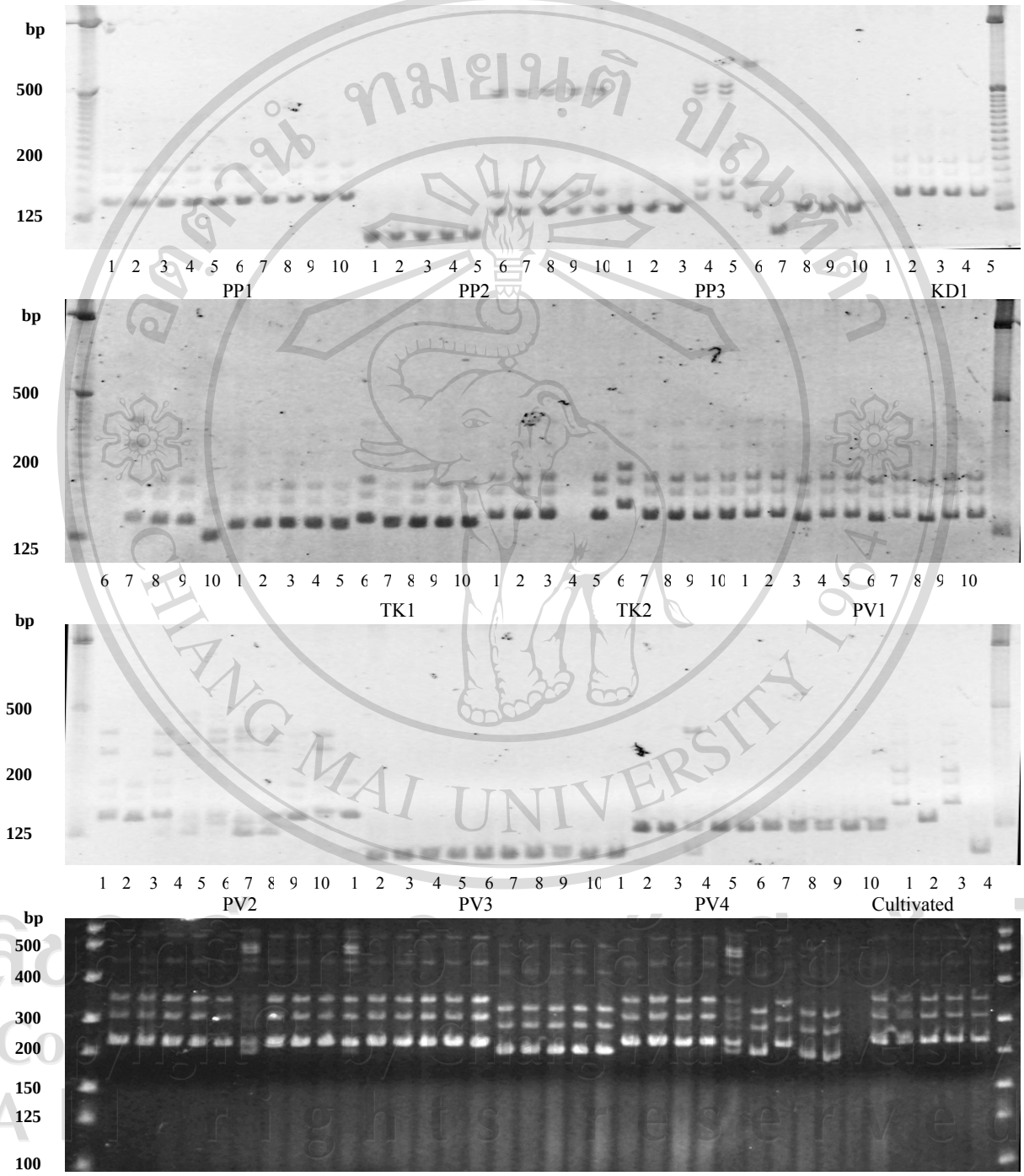
PP = Phnom Penh, KD = Kandal, TK = Takeo, PV = Prey Veng, ChP = Chhmar Prom, SK = Srau Krahorm, PhK = Phkar Khgney, ChR = Chomkoum Rumpak, SA = Sombok Angkrorng, MM = Mong Mang.

Appendix 8 Band patterns of 10 wild rice populations and 7 local rices at RM225 lucus.



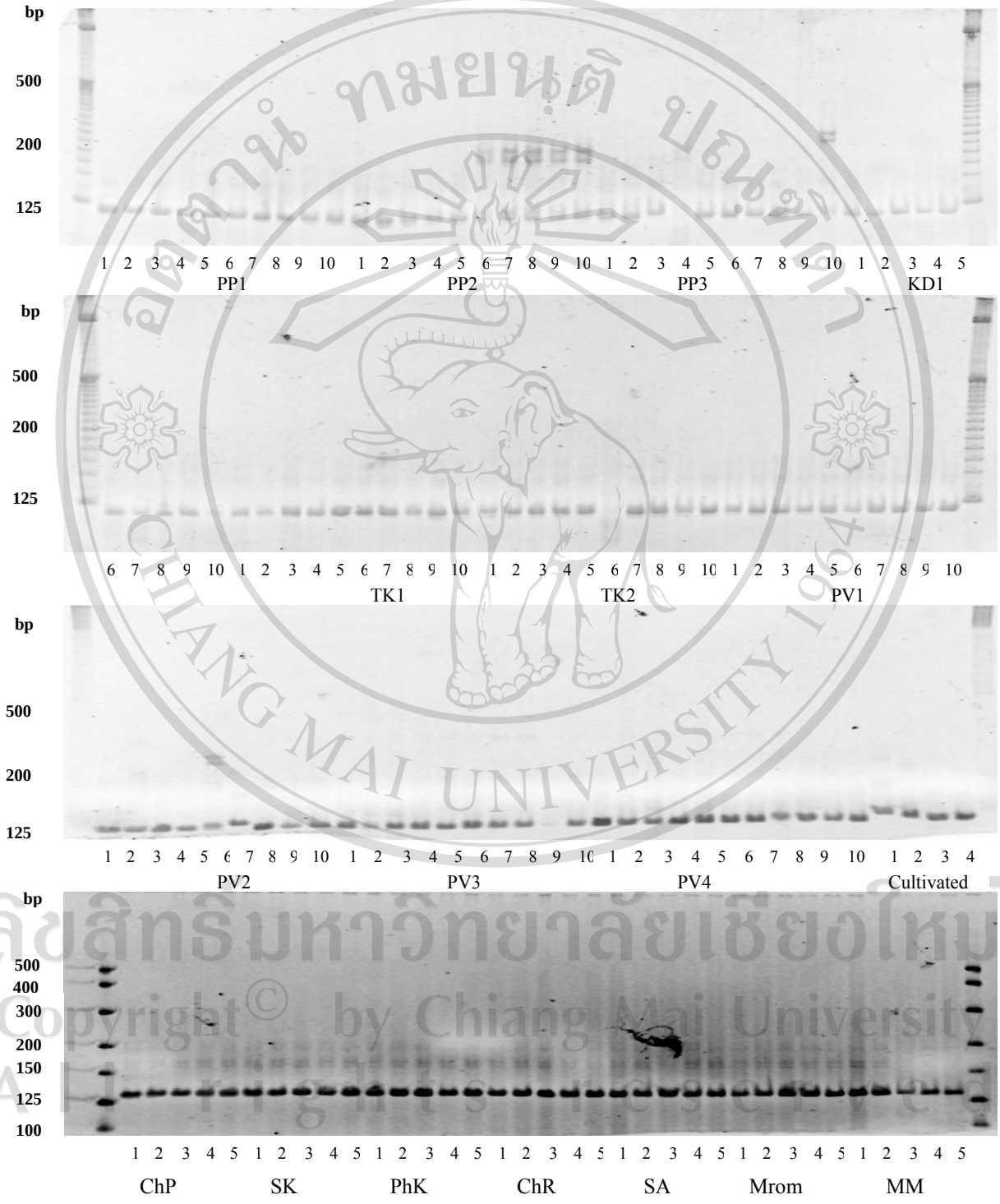
PP = Phnom Penh, KD = Kandal, TK = Takeo, PV = Prey Veng, ChP = Chhmar Prom, SK = Srau Krahorm, PhK = Phkar Khgney, ChR = Chomkoum Rumpak, SA = Sombok Angkrorng, MM = Mong Mang.

Appendix 9 Band patterns of 10 wild rice populations and 7 local rices at RM341 lucus.



PP = Phnom Penh, KD = Kandal, TK = Takeo, PV = Prey Veng, ChP = Chhmar Prom, SK = Srau Krahorm, PhK = Phkar Khgney, ChR = Chomkoum Rumpak, SA = Sombok Angkrorng, MM = Mong Mang.

Appendix 10 Band patterns of 10 wild rice populations and 7 local rices at RM588 lucus.



PP = Phnom Penh, KD = Kandal, TK = Takeo, PV = Prey Veng, ChP = Chhmar Prom, SK = Srau Krahorm, PhK = Phkar Khgney, ChR = Chomkoum Rumpak, SA = Sombok Angkrorng, MM = Mong Mang.

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