



APPENDIX

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่

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APPENDIX A

Reagents preparation

1% Agarose gel with ethidium

Agarose powder	1.0	g
1X TBE buffer	100.0	ml
Ethidium bromide	7.0	μ l

Digestion buffer :

2M Nacl (116.9 mg/ml)	5.0	ml
1M Tris pH 8.0 (121.1 mg/ml)	5.0	ml
0.5M EDTA (186.1 mg/ml)	0.2	ml
Millipore Water added to	100.0	ml

10 mM dNTPs solution:

100 mM dATP	0.1	ml
100 mM dGTP	0.1	ml
100 mM dCTP	0.1	ml
100 mM dTTP	0.1	ml
Millipore Water added	1.0	ml

0.5M EDTA pH 8.0 :

EDTA (Na ₂ ·2H ₂ O)	186.1
Millipore Water added	1000.0 ml
Adjust to pH	

Ethidium bromide solution :

Ethidium bromide	1.0 g
Millipore Water	100.0 ml

Phosphate buffer saline (PBS) pH 7.4 :

Sodium chloride	8766.0 mg
di-Sodium hydrogen phosphate	1495.0 mg
Potassium hydrogen phosphate	204.0 mg
Potassium chloride	200.0 mg
Millipore Water added to	1000.0 ml
Adjust to pH 7.4	
Autoclave	

3M Sodium acetate pH 5.2:

Sodium acetate (MW 82.03)	246.1 g
Millipore Water added to	1000.0 ml
Adjust to pH 5.2	

2M Sodium chloride:

Sodium chloride (MW 58.44)	116.9 g
Millipore Water added to	1000.0 ml

6M Sodium chlore :

Sodium chloride (MW 58.44)	350.6 g
Millipore Water added to	1000.0 ml

9% Sodium chloride :

Sodium chloride (MW 58.44)	9.0 g
Millipore Water	1000.0 ml

10% Sodium dodecyl sulfate (SDS):

Sodium dodecyl sulfate	10.0 g
Millipore Water	100.0 ml

1X TBE buffer:

10X TBE buffer	20.0 ml
Millipore Water added to	1000.0 ml

10X TBE buffer:

Tris	108.0 g
Boric acid	55.0 g

0.5M EDTA (186.1 mg/ml)	2.0 ml
Millipore Water added to	1000.0 ml

TE buffer:

1M Tris pH 8.0 (121.1 mg/ml)	10.0 ml
0.5M EDTA (186.1 mg/ml)	2.0 ml
Millipore Water added to	1000.0 ml

1M Tris pH 8.0:

Tris base	121.0 g.
Millipore Water added to	1000.0 ml

APPENDIX B

LOCUS EF512167 3098 bp DNA linear VRT 17-AUG-2011

DEFINITION *Oreochromis niloticus* anti-mullerian hormone gene (amh), complete cds.

ACCESSION EF512167

VERSION EF512167.1 GI:154101391

SOURCE *Oreochromis niloticus* (Nile tilapia)

ORGANISM [Oreochromis niloticus](#)

Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;

Actinopterygii; Neopterygii; Teleostei; Neoteleostei;

Acanthomorphata; Ovalentaria; Cichlomorphae; Cichliformes;

Cichlidae; African cichlids; Pseudocrenilabrinae; Oreochromini;

Oreochromis.

REFERENCE 1 (bases 1 to 3098)

AUTHORS Poonlaphdecha,S., Pepey,E., Huang,S.H., Canonne,M., Soler,L., Mortaji,S.,
Morand,S., Pfennig,F., Melard,C.,
Baroiller,J.F. and D'Cotta,H.

TITLE Elevated amh gene expression in the brain of male tilapia
(*Oreochromis niloticus*) during testis differentiation

JOURNAL Sex Dev 5 (1), 33-47 (2011)

PUBMED [21178329](#)

REFERENCE 2(bases 1 to 3098)

AUTHORS Pfennig,F., Standke,A., Ketscher,L., Storch,K. and Gutzeit,H.O.

TITLE Expression of amh (anti-mullerian hormone) in *Oreochromis niloticus*
during sexual differentiation and reproduction

JOURNAL Unpublished

REFERENCE 3 (bases 1 to 3098)

AUTHORS Pfennig,F., Standke,A., Ketscher,L. and Storch,K.

TITLE Direct Submission

ORIGIN

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1 aacatgttgg gtctgctcgt tctttacagc gaggcgctga cactctgctg gaccctgcaa
61 ccggcccagg accccacagt aaccggtcag acgccgctca gctgattaat taataactcg
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2761 actgcgggcc accagagcag accccagttc gtcagtgagg ggcggcgtct gtgggctgaa
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3061 tgcgggttgc agggagtgtg gatgccgcta gagctgct

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APPENDIX C

Statistic analysis

Crosstabs

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
snp1899 * sex	93	100.0%	0	0.0%	93	100.0%
snp2160 * sex	93	100.0%	0	0.0%	93	100.0%
snp2708 * sex	93	100.0%	0	0.0%	93	100.0%

SNP 1899 * sex

Crosstab

Count		sex		Total
		Male	Female	
SNP 1899	CC	32	16	48
	GC	16	23	39
	GG	1	5	6
Total		49	44	93

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Monte Carlo Sig. (2-sided)	
				Sig.	95% Confidence Interval Lower Bound
Pearson Chi-Square	9.014 ^a	2	.011	.007 ^c	.005
Likelihood Ratio	9.342	2	.009	.019 ^c	.017
Fisher's Exact Test	8.800			.009 ^c	.007
Linear-by-Linear Association	8.914 ^d	1	.003	.003 ^c	.002
McNemar-Bowker Test	.	.	. ^b		
N of Valid Cases	93				

SNP 2160 * sex

Crosstab

Count		sex		Total
		Male	Female	
SNP 2160	0	32	17	49
	1	16	23	39
	2	1	4	5
Total		49	44	93

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Monte Carlo Sig. (2-sided)	
				Sig.	95% Confidence Interval Lower Bound
Pearson Chi-Square	7.401 ^a	2	.025	.018 ^c	.015
Likelihood Ratio	7.588	2	.023	.049 ^c	.045
Fisher's Exact Test	7.209			.019 ^c	.016
Linear-by-Linear Association	7.308 ^d	1	.007	.009 ^c	.007
McNemar-Bowker Test	.	.	.	^b	
N of Valid Cases	93				

SNP 2708 * sex

Crosstab

Count		sex		Total
		Male	Female	
SNP	CC	39	24	63
2708	CT	10	20	30
Total		49	44	93

Chi-Square Tests^d

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1- sided)
Pearson Chi-Square	6.655 ^a	1	.010	.014	.009
Continuity Correction ^b	5.558	1	.018		
Likelihood Ratio	6.735	1	.009	.014	.009
Fisher's Exact Test				.014	.009
Linear-by-Linear Association	6.584 ^e	1	.010	.014	.009
McNemar Test				. ^c	
N of Valid Cases	93				

Measure of Linkage disequilibrium (LD)

> LD (SNP1,SNP2)

Pairwise LD

D	D'	Corr
Estimates: 0.2036721	0.9996084	0.9996084

X ²	P-value	N
LD Test: 185.8543	0	93

> LD (SNP2,SNP3)

Pairwise LD

D	D'	Corr
Estimates: 0.08956032	0.7765486	0.539454

	X²	P-value	N
LD Test:	54.12808	1.878497e-13	93

> **LD (SNP2,SNP3)**

Pairwise LD

D	D'	Corr
Estimates: 0.08956032	0.7765486	0.5394545

	X²	P-value	N
LD Test:	54.12808	1.878497e-13	93

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CURRICULUM VITAE

Name Miss Sawichaya Rueangsri

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Educations

- Dara Academy School, Chiang Mai, Thailand, 1999 - 2005: Certificated of Matayom VI
- Chiang Mai University, Chiang Mai, Thailand, 2005- 2009: Bachelor of Science (Agriculture) in Animal Sciences

Journals

2013 Poster presentation: Molecular genetic investigations of the AMH gene as candidate gene for temperature-dependent sex reversal in Nile tilapia (*Oreochromis niloticus*). *Agricultural Sci. J.* 44 : 1 (Suppl.) : 331-334 (2013)

