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APPENDICES

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Appendix 1 The information and manipulation of workplace for microinjection

The workplace for microinjection which consists of microinjector eppendorf 5242, micromanipulator MR and a Zeiss inverted microscope eases the injection into cells. A microscope is necessary to observe the cells and visually control the microinjection process. A micromanipulator with high positioning accuracy is required to bring microinstruments-microcapillaries. A microinjection is needed for the injection into a cell. The microinjection system consists of a pressure supply utilizing microelectronics for precision pneumatics and highly sensitive sensors, a capillary holder, and a drawn glass capillary. Minute volumes down to the picoliter range can be injected into living cells. A microcapillary filled with the injection fluid is connected with the microinjector proper via outlet and tube. The pressure gas (N_2 or air) supply by the system is adjustable in three independent pressure steps for injection, holding and cleaning. The capillary is positioned and the injection relaxed by a padal switch. Three variable pressure steps for different injection media and cell types as following:

1. Holding pressure (20-700 hPa, 0.02-0.7 bar) ; A constant which prevents cell matter from entering the capillary, and avoids clogging.

2. Injection pressure (40-2000 pa, 0.040-3 bar) ; When the capillary has penetrated the cell wall the permanently available holding pressure is raised to the injection pressure by pedal-switch operation. After injection the pressure automatically drops to the original level, variable injection time for 0.1-9.9 sec to obtain reproducible results.

3. Cleaning pressure (3000-7000 hPa, 3-7 bar) ; High pressure mainly used to blow out the microcapillary. It also eases filling of the capillary.

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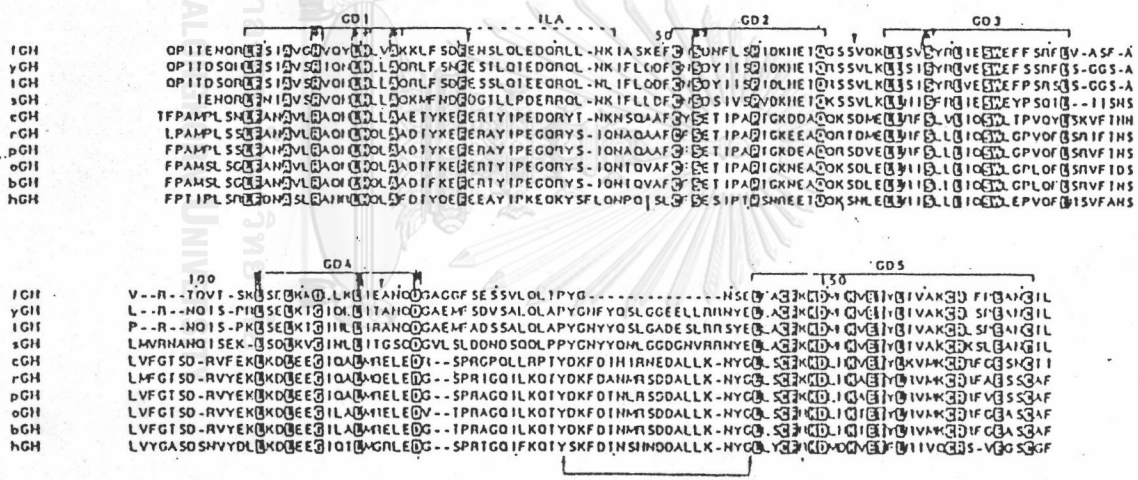
Appendix 2 Comparison of conserved amino acids and domains in mature growth hormone of various vertebrate (Watahiki and Yamamoto, 1989)



Appendix 3. Calculation of molecules number of plasmid

General formula:

1 mole	=	$6.02 * 10^{23}$	molecules (copies)
(duplex DNA) 1 Kb of DNA	=	$6.6 * 10^{5}$	daltons
(single strand DNA)	=	$3.3 * 10^{5}$	daltons

Calculating plasmid

designation of amount of plasmid	=	10^{6}	copies
10^{6} copies	=	$1.66 * 10^{-8}$	mole
weight	=	7.34	pg

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Appendix 4. Calculation of the annealing temperature (Tm) of primer

$$Tm = [\,2 * (A\text{-}T) + 4 * (G\text{-}C)\,]\ ^{0}C$$

hGH primers

Sense strand primer : 5'-CTGACCCAAGAGAACTCAC-3'

$$Tm = 58\ ^{0}C$$

Antisense strand primer ; 5'-CCCAGTCCGGGGGCTG-3'

$$Tm = 58\ ^{0}C$$

P. sutchi primers

Sense strand primer : 5'-CCCTTCGAGGATTTCTAC-3'

$$Tm = 54\ ^{0}C$$

Antisense strand primer ; 5'-CTACAGGGTGCAGTTGGA-3'

$$Tm = 56\ ^{0}C$$

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Appendix 5. Statistical analysis of comparison on hatching rate between microinjected egg and control (uninjected egg)

Analysis of Variance Procedure

Dependent Variable: HATCHING

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	18	32628.95050	1812.71947	9.20	0.0001
Error	42	8278.55863	197.10854		
Corrected Total	60	40907.50912			

Source	DF	Anova SS	Mean Square	F Value	Pr >F
REPLICA	15	30639.07689	2042.60513	10.36	0.0001
CELL	3	1989.87361	663.29120	3.37	0.0273

Duncan's Multiple Range Test for variable: HATCHING

Alpha= 0.05 df= 42 MSE= 197.1085

Harmonic Mean of cell sizes = 15.20362

Number of Means	2	3	4
Critical Range	10.28	10.80	11.16

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Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	CELL
A	40.668	16	control
B	30.223	14	4 cell
B	29.707	15	2 cell
B	25.912	16	1 cell

Appendix 6. Statistical analysis of comparison on survival rate of fry at one month old between microinjected fry and control (uninjected egg)

Analysis of Variance Procedure

Dependent Variable: SURVIVAL

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	12	13826.82907	1152.23576	2.90	.0157
Error	21	8331.66313	396.74586		
Corrected Total	33	22158.49220			

Source	DF	Anova SS	Mean Square	F Value	Pr > F
REPLICA	9	13096.37262	1455.15251	3.67	0.0068
CELL	3	730.45645	243.48548	0.61	0.6137

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Appendix 7. Linear regression analysis estimated growth rate of fish derived from microinjection at one cell stage egg and control (uninjected egg)

---------- Treat = control ----------

Dependent Variable: WEIGHT

Parameter Estimates

Variable	DF	Parameter estimate	Standard error	T for H0: Parameter=0	Prob > |T|
Intercep	1	-6.468294	1.57239799	-4.114	0.0001
Day	1	0.184678	0.02289189	8.067	0.0001

---------- Treat = one-cell micorinjected fish----------

Dependent Variable: WEIGHT

Parameter Estimates

Variable	DF	Parameter estimate	Standard error	T for H0: Parameter=0	Prob > |T|
Intercept	1	-10.581191	1.52113274	-6.956	0.0001
Day	1	0.276435	0.02117986	13.052	0.0001

Test: Numerator: 318.8126 DF: 1 F value: 4.8514

Denominator: 65.71513 DF: 375 Prob>F: 0.0282

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Appendix 8. Linear regression analysis estimated growth rate of fish derived from microinjection at two cell stage egg and control (uninjected egg)

---------- Treat = control ----------

Dependent Variable: WEIGHT

Parameter Estimates

Variable	DF	Parameter estimate	Standard error	T for H0: Parameter=0	Prob > |T|
Intercept	1	-5.414219	1.20627345	-4.488	0.0001
Day	1	0.147442	0.01674534	8.805	0.0001

---------- Treat = two-cell microinjected fish ----------

Dependent Variable: WEIGHT

Parameter Estimates

Variable	DF	Parameter estimate	Standard error	T for H0: Parameter=0	Prob > |T|
Intercept	1	-8.656475	1.97500300	-4.383	0.0001
Day	1	0.218433	0.02450850	8.913	0.0001

Test: Numerator: 82.6374 DF: 1 F value: 2.9123

Denominator: 28.37546 DF: 117 Prob>F: 0.0906

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Appendix 9. Linear regression analysis estimated growth rate of fish derived from microinjection at four cell stage egg and control (uninjected egg)

---------- Treat = control ----------

Dependent Variable: WEIGHT

Parameter Estimates

Variable	DF	Parameter estimate	Standard error	T for H0: Parameter=0	Prob > |T|
Intercep	1	-2.307651	2.60163766	-0.887	0.3835
Day	1	0.111202	0.03198663	3.477	0.0019

---------- Treat = four-cell microinjected fish ----------

Dependent Variable: WEIGHT

Parameter Estimates

Variable	DF	Parameter estimate	Standard error	T for H0: Parameter=0	Prob > |T|
Intercep	1	-0.668650	2.10071806	-0.318	0.7529
Day	1	0.088976	0.02582792	3.445	0.0020

Test: Numerator: 0.0067 DF: 1 F value: 0.0003

Denominator: 23.15911 DF: 51 Prob>F: 0.9865

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BIOGRAPHY

Miss. Surintorn Boonanuntansarn was born on Nevember 20, 1968, in Cholburi Province. She graduated with the Bachelor degree ((B.S.), cum laude.) in Aquatic science from Department of Aquatic Science, Faculty of Science, Burapha University in 1991. She continued study in Master degree Biotechnology Program, Faculty of Science, Chulalongkorn University.

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