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APPENDICES

APPENDIX A
ORIGINAL DATA FROM EXPERIMENT IN CHAPTER V

Table 1A The accuracy of competitive ELISA for determination of vitellogenin content in 2 haemolymph samples.

Haemolymph sample No. 1

		Well No.												AVG	SD	%
		1	2	3	4	5	6	7	8	9	10	11	12			
Plate No.	1	4.693	4.383	4.073	4.209	4.022	4.104	5.137	4.562	4.562	4.482	4.370	4.381	4.415	0.310	7.032
	2	4.694	4.286	3.868	4.063	3.987	4.377	5.291	4.494	5.015	4.742	4.210	3.972	4.417	0.445	10.079
	3	5.267	4.865	4.300	4.240	4.162	4.381	5.469	5.331	4.635	4.496	4.212	4.434	4.649	0.470	10.100
	4	5.165	4.593	4.550	4.403	4.508	4.680	5.104	4.230	4.899	4.606	5.316	5.002	4.755	0.336	7.069
	5	4.941	4.824	4.331	4.479	4.290	4.489	5.098	4.665	4.710	5.061	4.677	4.533	4.675	0.267	5.703
	6	4.660	4.400	4.840	4.920	4.847	5.110	4.657	4.825	4.847	4.006	4.884	4.106	4.675	0.337	7.208
	7	4.370	4.360	4.750	4.750	4.960	5.030	4.847	4.239	4.657	4.825	4.847	4.884	4.710	0.254	5.400
	8	4.560	4.470	4.740	4.650	4.760	4.940	4.657	4.884	4.006	4.847	4.884	4.295	4.641	0.275	5.930
	9	4.270	4.240	4.640	4.770	4.657	5.020	3.510	4.610	4.529	4.847	4.304	4.073	4.456	0.407	9.126
	10	4.500	4.370	4.390	4.720	4.840	4.950	3.648	3.755	4.035	4.261	4.292	4.205	4.330	0.397	9.173
														4.572	0.151	3.301

Haemolymph sample No. 2

		Well No.												AVG	SD	%
		1	2	3	4	5	6	7	8	9	10	11	12			
Plate No.	1	0.732	0.758	0.868	0.823	0.887	0.778	0.756	0.773	0.769	0.871	0.880	0.853	0.812	0.057	7.002
	2	0.648	0.633	0.847	0.860	0.826	0.850	0.843	0.823	0.806	0.832	0.831	0.868	0.806	0.079	9.800
	3	0.707	0.653	0.819	0.826	0.944	0.868	0.819	0.785	0.868	0.887	0.815	0.860	0.821	0.079	9.575
	4	0.721	0.703	0.774	0.758	0.838	0.958	0.823	0.904	0.930	0.930	0.868	0.877	0.840	0.086	10.191
	5	0.725	0.759	0.757	0.775	0.847	0.908	0.722	0.809	0.843	0.800	0.917	0.910	0.814	0.071	8.694
	6	0.906	0.831	0.775	0.854	0.796	0.852	0.962	0.938	0.912	0.911	0.866	0.802	0.867	0.060	6.866
	7	0.812	0.794	0.732	0.962	0.938	0.912	0.774	0.725	0.808	0.911	0.923	0.923	0.851	0.085	10.042
	8	0.846	0.817	0.778	0.831	0.779	0.868	0.825	0.825	0.849	0.826	0.850	0.751	0.820	0.034	4.197
	9	0.899	0.862	0.789	0.788	0.774	0.815	0.819	0.818	0.818	0.860	0.773	0.796	0.817	0.039	4.725
	10	0.908	0.866	0.802	0.792	0.794	0.911	0.923	0.923	0.924	0.908	0.722	0.809	0.825	0.070	8.435
														0.827	0.019	2.341

Table 2A Haemolymph vitellogenin levels of individual *P. monodon* at various stages of ovarian development.

No.	Body Weight (g)	Ovarian Weight (g)	OI (%)	Stages of Ovary	Ovarian Color	Vitellogenin Levels (mg/ml)
1	97.5	0.89	0.91	I	Transparence	0.000
2	89.7	0.61	0.68	I	"	0.000
3	111.8	0.95	0.85	I	"	0.000
4	114.76	6.42	5.59	IV	Grayish green	4.536
5	89.25	3.23	3.62	III	Green	1.547
6	74.74	2.02	2.70	II	Green	1.879
7	82.4	2.62	3.18	III	Grayish green	6.009
8	74.74	0.77	1.03	I	White	0.000
9	144.54	6.24	4.32	IV	Brown	0.515
10	168.28	5.68	3.38	III	Orange	0.206
11	136.19	3.30	2.42	II	Grayish green	5.549
12	154.06	3.90	2.53	II	Yellow	0.515
13	128.09	5.85	4.57	III	Yellow	7.002
14	84.62	3.38	3.99	III	Green	4.814
15	99.33	3.17	3.19	III	Gray	1.926
16	75.23	2.79	3.71	III	Gray	1.409
17	133.99	3.80	2.84	II	Yellow	5.057
18	73.05	0.76	1.05	I	White	0.000
19	88.54	3.42	3.86	III	Gray	1.645
20	76.56	1.74	2.27	II	Bright green	2.333
21	88.58	2.79	3.15	III	Gray	1.356
22	89.67	4.26	4.75	IV	Gray	2.028
23	87.54	2.83	3.23	III	Dark green	0.372
24	61.26	1.57	2.56	II	Gray	2.051
25	82.78	2.43	2.94	III	Gray	0.079
26	123.98	7.16	5.78	IV	Brown	0.867

Table 2A (Continued)

No.	Body Weight (g)	Ovarian Weight (g)	OI (%)	Stages of Ovary	Ovarian Color	Vitellogenin Levels (mg/ml)
27	70.02	2.69	3.84	III	Gray	0.064
28	78.12	0.42	0.54	I	Transparence	0.000
29	77.65	0.43	0.55	I	Transparence	0.000
30	118.32	1.09	0.92	I	"	0.000
31	68.85	0.41	0.60	I	"	0.000
32	119.35	0.8	0.67	I	"	0.000
33	85.26	0.65	0.76	I	"	0.000
34	112.4	0.6	0.53	I	"	0.000
35	87.61	0.36	0.41	I	"	0.000
36	88.61	0.54	0.61	I	"	0.000
37	89.91	0.5	0.56	I	"	0.000
38	67.37	0.32	0.47	I	"	0.000
39	84.81	0.36	0.42	I	"	0.000
40	93.70	4.00	4.27	III	Green	11.752
41	78.10	2.30	2.94	Spent	Yellow	1.338
42	119.50	4.50	3.77	III	Green	1.300
43	101.48	3.52	3.47	IV	Green	0.022
44	102.76	1.85	1.80	I	Transparence	0.013
45	101.34	4.20	4.14	IV	Grayish green	2.326
46	63.20	0.80	1.27	I	Transparence	0.000
47	87.60	3.10	3.54	IV	Bright green	0.197
48	93.76	2.58	2.75	Spent	Yellow	0.512
49	138.56	4.55	3.28	III	Bright green	0.965
50	129.96	2.22	1.71	II	White	0.317
51	131.27	1.75	1.33	II	"	0.385
52	148.19	1.88	1.27	II	"	0.424

Table 2A (Continued)

No.	Body Weight (g)	Ovarian Weight (g)	OI (%)	Stages of Ovary	Ovarian Color	Vitellogenin Levels (mg/ml)
53	132.79	2.45	1.85	II	Yellow	1.143
54	111.14	1.77	1.59	II	Yellow	0.363
55	140.18	5.68	4.05	IV	Grayish green	0.793
56	139.09	2.32	1.67	I	Transparence	0.000
57	141.80	3.05	2.15	II	White	0.289
58	159.26	2.22	1.39	II	"	0.164
59	220.00	3.20	1.45	II	Yellow	0.145
60	126.65	4.66	3.68	III	Gray	11.228
61	105.96	1.38	1.30	II	Yellow	0.177
62	123.38	4.88	3.96	III	Yellow	1.383
63	155.82	3.42	2.19	Spent	White	2.839
64	115.78	9.30	8.03	IV	Yellowish green	2.025
65	113.70	3.66	3.22	IV	Yellowish gray	0.296
66	163.69	7.49	4.58	IV	Yellow	1.074
67	127.69	4.24	3.32	Spent	Yellow	0.948
68	149.08	11.49	7.71	IV	Yellowish gray	1.728
69	111.38	3.98	3.57	III	Yellow	2.106
70	105.80	4.46	4.22	IV	Yellowish green	1.888
71	132.30	4.09	3.09	IV	Green	0.943
72	124.26	9.08	7.31	IV	Green	1.739
73	138.57	15.80	11.40	IV	Green	1.17
74	116.80	3.10	2.65	III	Green	0.124
75	103.40	2.00	1.93	II	Yellow	2.041

Table 3A The haemolymph vitellogenin levels after eye ablation in *P. monodon.*

No.	Vitellogenin Levels (mg/ml) from the beginning time after eye ablation (days)											OI
	0	2	3	4	5	6	7	8	9	10	11	12	(%)
1	0	1.659	1.661	2.610	0.572	0.584	0.471	3.447	3.086	1.350	1.604	2.025	8.03
2	0	0.667	1.397	1.425	0.330	0.399	0.379	1.231	1.831	0.471	0.519	0.296	3.22
3	0	2.114	1.825	1.544	0.202	0.550	0.976	3.010	2.781	1.610	1.453	1.074	4.58
4	0	0.755	1.306	0.657	0.252	0.127	0.140	1.460	3.416	1.260	1.341	0.948	3.32
5	0	1.530	1.429	1.829	0.326	0.398	0.343	1.093	2.484	1.210	2.122	1.728	7.71
6	0	0.998	1.078	1.054	0.589	1.728	1.819	0.895	1.449	1.862	3.276	2.106	3.57
7	0	1.677	1.546	0.895	0.061	0.078	0.424	2.044	3.455	1.083	1.352	1.888	4.22
Mean	0	1.343	1.463	1.431	0.333	0.552	0.650	1.883	2.643	1.264	1.667	1.438	4.95
SD	0	0.542	0.243	0.657	0.192	0.553	0.575	0.995	0.773	0.438	0.854	0.677	2.05

Table 4A Alteration of haemolymph vitellogenin levels in eye-ablated *P. monodon* after injection with 300 μl saline/prawn at 2-24 hr.

No.	Vitellogenin level at the beginning experiment	Alteration of vitellogenin levels (mg/ml) from the beginning time after injection (hr)						OI (%)
		2	4	6	10	14	24	
1	0.309	-0.046	0.047	0.110	0.792	0.293	0.237	2.76
2	0.313	-0.067	0.403	0.362	0.323	1.668	1.423	5.03
3	1.038	-0.200	-0.155	0.131	0.557	0.077	0.338	2.67
4	0.224	0.121	0.500	0.891	0.758	0.898	0.761	6.15
5	0.102	0.023	0.045	-0.036	-0.043	-0.042	-0.043	3.41
6	0.464	0.074	0.080	0.138	0.164	0.270	0.034	4.77
7	0.813	-0.421	-0.317	-0.222	0.283	0.930	1.926	3.02
8	0.507	0.385	0.637	0.305	0.183	0.629	0.256	4.51
9	0.381	0.089	0.132	0.050	0.152	0.347	0.192	1.64
Mean	0.461	-0.005	0.153	0.192	0.352	0.563	0.569	3.77
SD	0.295	0.224	0.309	0.314	0.288	0.534	0.678	1.425

Table 5A Alteration of haemolymph vitellogenin levels in eye-ablated *P. monodon* after injection with extract from 3 eyestalks/prawn at 2-24 hr.

No.	Vitellogenin level at the beginning experiment	Alteration of vitellogenin levels (mg/ml) from the beginning time after injection (hr)						OI (%)
		2	4	6	10	14	24	
1	1.034	0.763	1.830	1.329	1.321	1.203	1.098	4.91
2	0.481	0.680	1.329	0.827	1.017	0.722	0.597	2.8
3	0.155	1.382	3.145	3.951	3.453	2.673	2.581	3.23
4	0.615	0.315	1.444	1.441	1.644	1.717	1.942	4.34
5	0.447	0.506	0.970	0.909	1.384	1.157	1.277	4.97
Mean	0.546	0.729	1.744	1.691	1.764	1.495	1.499	4.05
SD	0.320	0.404	0.842	1.290	0.970	0.747	0.773	0.989

Table 6A Alteration of haemolymph vitellogenin levels in eye-ablated *P. monodon* after injection with extract from 2, 1 and 1/2 eyestalks/prawn at 2-10 hr.

2 eyestalks

No.	Vitellogenin level at the beginning experiment	Alteration of vitellogenin levels (mg/ml) from the beginning time after injection (hr)				OI (%)
		2	4	6	10	
1	0.407	0.335	0.776	0.919	1.033	3.10
2	0.654	0.305	0.549	0.851	0.756	7.00
3	0.592	0.630	2.613	3.049	3.363	9.03
Mean	0.551	0.423	1.313	1.607	1.718	6.38
SD	0.129	0.179	1.132	1.250	1.432	3.01

1 eyestalk

No.	Vitellogenin level at the beginning experiment	Alteration of vitellogenin levels (mg/ml) from the beginning time after injection (hr)				OI (%)
		2	4	6	10	
1	0.665	0.441	1.297	2.273	2.203	3.58
2	0.665	0.480	0.780	0.966	0.696	4.64
3	0.523	0.232	0.719	0.370	0.579	3.75
Mean	0.618	0.384	0.932	1.203	1.159	3.99
SD	0.082	0.133	0.318	0.974	0.906	0.57

1/2 eyestalk

No.	Vitellogenin level at the beginning experiment	Alteration of vitellogenin levels (mg/ml) from the beginning time after injection (hr)				OI (%)
		2	4	6	10	
1	0.429	0.011	0.443	0.543	0.486	3.59
2	0.489	0.143	0.168	0.143	0.197	2.00
3	0.563	0.242	0.812	0.503	0.801	7.04
4	0.568	-0.001	0.042	0.125	0.142	4.61
Mean	0.512	0.099	0.366	0.329	0.406	4.31
SD	0.066	0.115	0.341	0.225	0.303	2.12

APPENDIX B

BUFFER AND REAGENT PREPARATION

1. Phosphate buffered saline (PBS) 0.15 M pH 7.2 (Hudson and Hay. 1976)

NaCl	8.00	g
KCl	0.20	g
KH_2PO_4	0.20	g
Na_2HPO_4	1.15	g
or $Na_2HPO_4 . 7H_2O$	2.15	g
H_2O (distilled water) adjust volume to	1,000.0	ml

2. Bradford solution (Bradford. 1976)

Coomassie brilliant blue G-250	100.0	mg
Methanol	50.0	ml
85% Phosphoric acid	100.0	ml
H_2O (distilled water) adjust volume to	1,000.0	ml

3. 1% Merthiolate

Thimerosal (Sigma)	1.0	g
H_2O (distilled water) adjust volume to	100.0	ml

APPENDIX C

REAGENT PREPARATION FOR HYBRIDOMA PRODUCTION

1. RPMI medium

The medium consisted of the following ingredients :

Ingredient	Amount	Unit
RPMI 1640 (Roswell Park Memorial Institute - Gibco BRL, USA)	10.4	g
D-glucose (Sigma)	3.6	g
L-glutamine (Sigma)	0.2923	g
Sodium pyruvate ($C_3H_3O_3Na$) (Sigma)	1.1005	g
$NaHCO_3$	2.0160	g
HEPES (N-2-Hydroxyethylpiperazine-N'-2-ethanesulfonic acid, Sigma)	5.9525	g
H_2O (Meri Q water)	1,000.0	ml

The solution of penicillin G, streptomycin and kanamycin were added to the final concentration of 20,000 units, 200 mg and 200 mg per liter, respectively. The medium was sterilized by millipore membrane (pore size 0.22 μm) filtration and stored at 4 °C.

2. RPMI medium with serum

The medium consisted of the following ingredients :

Ingredient	Amount	Unit
RPMI medium (1)	80.0	ml
Fetal calf serum (FCS, Starrate, Australia) or Bovine calf serum (BCS, Starrate, Australia)	20.0	ml
100 X HT supplement (Gibco BRL, USA) -10 mM sodium hypoxanthine -1.6 mM thymidine	1.0	ml

3. Hybridoma selective medium (HAT medium)

The medium consisted of the following ingredients :

Ingredient	Amount	Unit
RPMI medium (1)	80.0	ml
FCS	20.0	ml

HT supplement	1.0	ml
50 X Aminopterin (Sigma)	2.0	ml
1 % Mouse red blood cell		

4. Fusion solution (40 % polyethylene glycol)

Polyethylene glycol (PEG)	4.0	g

The solution was prepared by dissolving 4.0 g of polyethylene glycol in 6 ml of RPMI medium (1). The solution was incubated at 37 °C in CO_2 incubator before use.

5. Freezing medium (12 % DMSO)

Dimethylsulfoxide (DMSO, Sigma)	12.0	ml
RPMI medium (1)	88.0	ml

The medium was stored in cold (4 °C) before use.

APPENDIX D

BUFFER AND SOLUTION FOR POLYACRYLAMIDE GEL ELECTROPHORESIS (PAGE), SODIUM DODECYL SULFATE POLYACRYLAMIDE GEL ELECTROPHORESIS (SDS-PAGE) AND WESTERN BLOT ANALYSIS

1. Stock solution :

1.1 Monomer solution (30 % T, 2.7 % C_{Bis})

Acrylamide (BIO-RAD)	58.4	g
Bis (N,N'-methylene-bis-acrylamide, BIO-RAD)	1.6	g
H_2O (distilled water) adjust volume to	200.0	ml

Stored at 4 °C in the dark bottle.

1.2 4 X Running gel buffer (1.5 M tris-Cl pH 8.8)

Tris (hydroxymethyl) aminomethane (BIO-RAD)	36.3	g
H_2O (distilled water) adjust volume to	200.0	ml

adjusted pH with HCl

1.3 4 X Stacking gel buffer (0.5 M tris-Cl pH 6.8)

Tris	3.0	g
H_2O (distilled water) adjust volume to	50.0	ml

adjusted pH with HCl

1.4 10 % SDS

SDS (sodium dodecyl sulfate, BIO-RAD)	50.0	g
H_2O (distilled water) adjust volume to	500.0	ml

1.5 10 % Ammonium persulfate (freshly prepared)

Ammonium persulfate (BIO-RAD)	0.1	g
H_2O (distilled water)	1.0	ml

1.6 Running gel overlay (0 375 M tris-Cl pH 8.8, 0.1 % SDS)

1.5 M Tris (1.2)	25.0	ml
10 % SDS (1.4)	1.0	ml
H_2O (distilled water) adjust to	100.0	ml

1.7 2 X Treatment buffer (0.125 M tris-Cl pH 6.8, 4 % SDS, 20 % glycerol, 10 % 2-mercaptoethanol)

0.5 M Tris (1.3)	2.5	ml
10 % SDS (1.4)	4.0	ml
Glycerol	2.0	ml
2-Mercaptoethanol	1.0	ml
H_2O (distilled water)	0.5	ml

The treatment buffer of PAGE had not SDS and 2-mercaptoethanol

2. Preparation of separating gel and stacking gel

2.1 Separating gel and stacking gel for PAGE 5 % gel (5 % T 2.7 % C_{Bis})

Monomer solution (1.1)	5.0	ml
1.5 M tris-Cl (1.2)	7.5	ml
H_2O (distilled water)	17.05	ml
10 % Ammonium persulfate (1.5)	150.0	µl
TEMED (BIO-RAD)	20.0	µl

2.2 Separating gel for SDS-PAGE 7.5 % gel (7.5 % T 2.7 % C_{Bis})

Monomer solution (1.1)	7.5	ml
1.5 M tris-Cl pH 8.8 (1.2)	7.5	ml
10 % SDS (1.4)	0.3	ml
H_2O (distilled water)	14.55	ml
10 % Ammonium persulfate (1.5)	150.0	µl
TEMED	20.0	µl

2.3 Stacking gel for SDS-PAGE 4 % gel (4 % T 2.7 % C_{Bis})

Monomer solution (1.1)	2.66	ml
0.5 M tris-Cl pH 6.8 (1.3)	5.0	ml
10 % SDS (1.4)	0.2	ml
H_2O (distilled water)	12.2	ml
10 % Ammonium persulfate (1.5)	100.0	µl
TEMED	10.0	µl

Table 1D Preparation of separating gel and stacking gel

	Separating gel		Stacking gel
	5 % T 2.7 % C_{Bis} (for PAGE)	7.5 % T 2.7 % C_{Bis} (for SDS-PAGE)	4 % T 2.7 % C_{Bis} (for SDS-PAGE)
30 % T 2.7 % C_{Bis} (1.1)	5 ml	7.5 ml	2.66 ml
1.5 M tris-Cl pH 8.8 (1.2)	7.5 ml	7.5 ml	-
0.5 M tris-Cl pH 6.8 (1.3)	-	-	5 ml
10 % SDS (1.4)	-	0.3 ml	0.2 ml
H_2O	17.05 ml	14.55 ml	12.2 ml
Mixed and deaerated using vacuum pump			
10 % Ammonium persulfate (1.5)	150 µl	150 µl	100 µl
TEMED	20 µl	20 µl	10 µl
Mixed and rapidly poured between the glass plate			

3. Running buffer :

3.1 PAGE -Upper tank buffer (37.6 mM tris, 40 mM HCl, pH 8.89)

Tris	4.56	g
Glycine (BIO-RAD)	3.0	g
H_2O (distilled water) adjust to	1,000.0	ml

3.2 PAGE -Lower tank buffer (63 mM tris, 50 mM HCl, pH 7.47)

Tris	22.7	g
1 N HCl	150.0	ml
H_2O (distilled water) adjust to	3,000.0	ml

3.3 SDS-PAGE Tank buffer

(0.025 M tris pH 8.3, 0.192 M glycine, 0.1 % SDS)

Tris	12.0	g
Glycine	57.6	g
10% SDS (1.4)	40.0	ml
H_2O (distilled water)	4,000.0	ml

4. Staining and destaining solution

4.1 Staining solution for protein (Coomassie blue)

4.1.1 Stain stock (1% Coomassie blue R-250)

1% Coomassie blue R-250	1.0	g
H_2O (distilled water)	100.0	ml

4.1.2 Stain (0.1 % Coomassie blue R-250, 50% methanol, 10% acetic acid)

Stain stock (4.1.1)	50.0	ml
Methanol	250.0	ml
Acetic acid	50.0	ml
H_2O (distilled water) adjust to	500.0	ml

4.2 Destaining solution for Coomassie blue

4.2.1 Destain I (50% methanol, 10% acetic acid)

Methanol	500.0	ml
Acetic acid	100.0	ml
H_2O (distilled water)	1,000.0	ml

4.2.2 Destain II (5% methanol, 7% acetic acid)

Methanol	50.0	ml
Acetic acid	70.0	ml
H_2O (distilled water)	1,000.0	ml

Method of gel staining for protein

A gel was stained with 0.1 % Coomassie blue R-250, 50% methanol, 10% acetic acid for 5-6 hrs. The gel was then washed in destain I for 1 hr. with 1-2 changes and followed by destain II until the gel was cleared. After washing in distilled H_2O for a few times, the gel was dried in a gel air dryer (BIO-RAD)

4.3 Staining solution for carbohydrate (Periodic Schiff reagent-PAS)

4.3.1 Periodic acid

Periodic acid (HIO_4, Sigma)	0.6	g
Nitric acid (concentrated)	0.3	ml
H_2O (distilled water)	100.0	ml

4.3.2 Schiff reagent

Basic fuchsin	0.5-1.0	g
Sodium metabisulfite ($Na_2S_2O_5$)	1.9	g
1 N HCl	15.0	ml
H_2O (distilled water)	85.0	ml

The solution was shaked at intervals for at least 2 hours or overnight. An activated charcoal was added for 1 minute and shaked occasionally. Filter. The solution was water-white.

4.3.3 Sodium metabisulfite solution

Sodium metabisulfite ($Na_2S_2O_5$)	0.5	g
H_2O (distilled water)	100.0	ml

Method of gel staining for carbohydrate (modified from Humason, 1979)

A gel was fixed in 10 % formalin overnight and washed in distilled H_2O for 15 min, 4 times. The gel was put in Periodic acid for 30-40 min and washed as above. The gel was then put in Schiff reagent overnight and washed in sodium metabisulfite solution until the gel was cleared. After washing in distilled H_2O for a few time, the gel was dried as above.

4.4 Stain solution for lipid (Sudan black B-SBB)

Sudan black B	0.7	g
Ethylene glycol	100.0	ml

4.5 Destaining solution for Sudan black B

(20 % acetone, 15 % acetic acid)

Acetone	20.0	ml
Acetic acid	15.0	ml
H_2O (distilled water)	65.0	ml

Method of gel staining for lipid (modified from Humason, 1979)

A gel was fixed in 10 % formalin overnight and washed in distilled H_2O for 15 min, 4 times. The gel was put in ethylene glycol for 20 min with 2 changes and put in SBB solution overnight. The gel was then washed in 20% acetone, 15 % acetic acid) until the gel was cleared. After washing in distilled H_2O for a few times, the gel was dried as above.

5. SDS molecular weight markers (Sigma) consist of :

-Myosin, Rabbit muscle	205	kD
-β Galactosidase, *Escherichia coli*	116	kD
-Phosphorylase B, Rabbit muscle	97.4	kD
-Albumin, Bovine plasma	66	kD
-Albumin, Egg (Ovalbumin)	45	kD
-Carbonic anhydrase (Bovine erythrocytes)	29	kD

6. Towbin transfer buffer pH 8.8 for Western blot analysis

(25 mM tris, 192 mM glycine, 20 % methanol)

The buffer consisted of the following ingredients :

Tris	3.03	g
Glycine	14.4	g
Meythanol	200.0	ml
H_2O (distilled water) adjusted to	1,000.0	ml

The buffer was pre-chill before use.

APPENDIX E

BUFFERS AND SOLUTION FOR ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA)

1. 5% Blotto solution (Johnson et al., 1984)

Skimmed milk	5.0	g
PBS 0.15 M pH 7.2	100.0	ml
1% Merthiolate (Sigma)	1.0	ml
Triton X-100 (Sigma)	0.1	ml

2. Washing solution (0.5 % Blotto)

5 % Blotto solution (4.1)	50.0	ml
PBS 0.15 M pH 7.2	950.0	ml

3. 0.1 M Citrate Buffer pH 4.5

Sodium citrate	29.41	g
1% Merthiolate	10.0	ml
H_2O (distilled water) adjust volume to	1,000.0	ml

The pH of the buffer was adjusted to 4.5 with 0.1 M HCl

4. 1 N H_2SO_4

Concentrated H_2SO_4	27.6	ml
H_2O (distilled water) adjusted volume to	1,000.0	ml

5. O-Phenylenediamine dihydrochloride (OPD)

APPENDIX F
REAGENT FOR DETERMINATION OF ISOTYPES AND SUBISOTYPES OF MONOCLONAL ANTIBODIES USING ANTIBODY CAPTURED ON ANTI-Ig ANTIBODIES

Hybridoma sub-isotyping kit, mouse (Zymed) contents :

A) Rabbit anti-Mouse IgG1 (γ_1 chain specific)

B) Rabbit anti-Mouse IgG2a (γ_{2a} chain specific)

C) Rabbit anti-Mouse IgG2b (γ_{2b} chain specific)

D) Rabbit anti-Mouse IgG3 (γ_3 chain specific)

E) Rabbit anti-Mouse IgA (α chain specific)

F) Rabbit anti-Mouse IgM (μ chain specific)

G) Rabbit anti-Mouse kappa light chain

H) Rabbit anti-Mouse lambda light chain

I) Normal Rabbit Serum, (Negative Control)

J) Positive Control, Monoclonal Mouse IgG1
(Mouse IgG1 is in RPMI-1640 with 10 % FBS)

K) Substrate Buffer, Cencentration (10 X)
(1 M citrate, pH 4.2, containing 0.03 % H_2O_2)

L) ABTS Substrate, Concentrated (50 X)
(2,2-azino-di[3-ethylbenzthiazoline sulfonic acid])

M) Blocking Solution, Concentration (50 X)
(25 % BSA in PBS and 0.05 % NaN_3)

N) HRP-Goat anti-Rabblt IgG (H+L), Concentrated (50 X)

O) Goat anti-Mouse IgGAM, Concentrated (50 X)
(0.5 mg/ml in PBS containing 10 % glycerol and 0.05 % NaN_3)

P) 50 % Tween 20

BIOGRAPHY

Mr. Siwaporn Longyant was born on November 21, 1968 in Bangkok. He graduated with a Bachelor Degree of Science (Biology) from Srinakharinwirot University, Prasarnmit campus in 1991 and Master Degree of Science (Biological chemistry) from Srinakharinwirot University, Prasarnmit campus in 1994.

He studied for the Degree of Doctor of Philosophy in Marine Science at the Department of Marine Science, Faculty of Science, Chulalongkorn University since 1996. During his study, he received certificate of award from the Congress on Science and Technology of Thailand for winning first place in the poster competition in Biochemistry Field during the 24th Congress on Science and Technology of Thailand (STT-24) (October 19-21, 1998 at Queen Sirikit National Convention Center, Bangkok) and received certificate of award from the Asian Fisheries Society for winning first place in the poster competition for student during the Fifth Asian Fisheries Forum : International Conference on Fisheries and Food Security Beyond the Year 2000 (November 11-14, 1998 at Chiang Mai, Thailand).

At present he is the lecturer of the Department of Biology, Faculty of Science, Srinakharinwirot University, Bangkok.

Research publications :

1. Monoclonal antibodies production specific to vitellin and vitellogenin of giant prawn *Penaeus monodon*. Invert. Reprod. Develop. 35:1 (1999) : 9-17.
2. Characterization of vitellin and vitellogenin of giant tiger prawn *Penaeus monodon* using monoclonal antibodies specific to vitellin subunits. Invert. Reprod. Develop. (in press).