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APPENDICES

APPENDIX A
SOLUTIONS AND REAGENTS PREPARATION

1. Preparation of Competent cell for CaCl₂-transformation

The 10 min pre-chilled culture was centrifuged at 4,000 x g for 5 min. Then, cells were washed once with 0.5 volume of 10 mM CaCl₂ solution. The final cell pellet was resuspended in an appropriate volume of 100 mM CaCl₂ solution supplemented with 10% (v/v) glycerol and chilled on ice for about 30 min. One hundred microliters of competent cells were aliquoted into new eppendorf tubes and immediately frozen at -80 °C until used.

2. Reagent setup

URA media

2.0 g/l of yeast synthetic drop-out medium without Uracil

6.7 g/l of yeast nitrogen base without amino acids

2% glucose (for pre-culture) or 0.1% glucose (expression culture)

2% glucose and add bacteriological agar 20 g/l (for plates)

YSB

50 mM Tris-HCl (pH 7.6)

5 mM EDTA

10% glycerol

1X complete protease inhibitor cocktail tablets.

YPD media

10 g/l of bacto-yeast extract

20 g/l of bacto-peptone

20 g/l of dextrose



PBS (1 litre)

1.44 g of $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ (8.1 mM)

0.25 g of KH_2PO_4 (1.9 mM)

8.00 g of NaCl

0.2 g of KCl

Adjust pH to 7.4 using 1 M NaOH or 1 M HCl

Adjust final volume to 1 litre with distilled water.

3. Preparation for SDS-PAGE electrophoresis**Stock reagents****30% Acrylamide. 0.8% bis-acrylamide (100 ml)**

29.2 g of Acrylamide

0.80 g of N,N'-methylene-bis-acrylamide

Adjust final volume to 100 ml with distilled water.

1.5 M Tris-HCl pH 8.8

18.17 g of Tris-HCl

Adjust pH to 8.8 with 1 M HCl

Adjust volume to 100 ml with distilled water.

1.0 M Tris-HCl pH 6.8

12.1 g of Tris-HCl

Adjust pH to 6.8 with 1 M HCl

Adjust volume to 100 ml with distilled water.



SDS-PAGE**15% Seperating gel**

Distilled water 2.26 ml
1.5 M Tris-HCl (pH 8.8) 2.53 ml
30% Acrylamide ml solution 5 ml
10% SDS 100 μ l
10% APS, $(\text{NH}_4)_2\text{S}_2\text{O}_8$ 100 μ l
TEMED 4 μ l

5.0% Stacking gel

Distilled water 2.75 ml
1.0 M Tris-HCl (pH 6.8) 0.50 ml
30% Acrylamide ml solution 0.67 ml
10% SDS 40 μ l
10% APS, $(\text{NH}_4)_2\text{S}_2\text{O}_8$ 40 μ l
TEMED 4 μ l

5X Sample buffer

1 M Tris-HCl (pH 6.8) 0.6 ml
50% Glycerol 5.0 ml
10% SDS 2.0 ml
2-Mercaptoethanol 0.5 ml
1% Bromophenol blue 1.0 ml
Distilled water 0.9 ml

One part of sample buffer was added to four parts of sample. The mixture was heated 5 min. in boiling water before loading to the gel.



Electrophoresis buffer (1X), 1 litre

3.03 g of Tris-HCl

14.40 g of Glycine

1.0 g of SDS

Dissolve in distilled water to 1 litre.

Do not adjust pH with acid or base (final pH should be 8.3).



APPENDIX B

CIRCULAR DICHROISM SPECTROSCOPY

Circular dichroism studies were carried out with a JASCO J-715 CD Spectropolarimeter with temperature controller using a 1 mm optical cuvette. Spectra were recorded between 180-320 nm, using 0.42 mg/ml purified *rPmVRP15* proteins in 10 mM Phosphate buffer pH 7.0. Each spectrum was the average of three scans and background was subtracted with the spectrum of 10 mM Phosphate buffer pH 7.0 (blank). The CD spectra of purified *rPmVRP15* proteins in 10 mM phosphate buffer pH 7.0. was measured. The results were presented as mean residue ellipticity $[\theta]$ in $\text{deg cm}^2 \text{dmol}^{-1}$, which is defined as

$$[\theta] = \theta / (10 \times n \times C_p \times l)$$

Where θ is the ellipticity in milli-degrees

n is the number of amino acid residues ((in atomic mass units/daltons)/ number of amino acid residues) = (15,899 Da/137 aa)

C_p is molar concentration of the protein. (M)

l is the cell path length (1 cm)

The data of mean residue ellipticity $[\theta]$ of *rPmVRP15* was calculated as table below.



Table 4.1 The data of mean residue ellipticity $[\theta]$ of rPmVRP15

Wavelength (nm)	CD (θ)	HT	MRW	$\theta/10$	a (cm.)	c (g/ml)	$[\theta]$ MRE, λ
180	5.315	842.901	116.904	0.532	1	0.00042	0.02610
181	8.929	727.178	116.904	0.893	1	0.00042	0.04384
182	12.598	651.437	116.904	1.260	1	0.00042	0.06185
183	14.589	607.464	116.904	1.459	1	0.00042	0.07163
184	16.543	583.326	116.904	1.654	1	0.00042	0.08123
185	19.792	570.759	116.904	1.979	1	0.00042	0.09718
186	22.575	565.208	116.904	2.257	1	0.00042	0.11084
187	25.082	563.639	116.904	2.508	1	0.00042	0.12315
188	27.761	563.814	116.904	2.776	1	0.00042	0.13631
189	30.557	564.724	116.904	3.056	1	0.00042	0.15003
190	33.048	565.310	116.904	3.305	1	0.00042	0.16227
191	35.060	565.135	116.904	3.506	1	0.00042	0.17214
192	36.480	563.490	116.904	3.648	1	0.00042	0.17911
193	37.117	560.272	116.904	3.712	1	0.00042	0.18225
194	36.724	555.206	116.904	3.672	1	0.00042	0.18032
195	35.607	548.111	116.904	3.561	1	0.00042	0.17483
196	33.000	539.253	116.904	3.300	1	0.00042	0.16203
197	28.674	528.601	116.904	2.867	1	0.00042	0.14079
198	23.400	516.770	116.904	2.340	1	0.00042	0.11489
199	17.180	504.584	116.904	1.718	1	0.00042	0.08435
200	10.506	492.302	116.904	1.051	1	0.00042	0.05158
201	4.413	480.395	116.904	0.441	1	0.00042	0.02167
202	-1.046	468.953	116.904	-0.105	1	0.00042	-0.00514
203	-5.693	458.199	116.904	-0.569	1	0.00042	-0.02795
204	-9.158	448.329	116.904	-0.916	1	0.00042	-0.04497
205	-12.099	439.133	116.904	-1.210	1	0.00042	-0.05940
206	-14.543	430.597	116.904	-1.454	1	0.00042	-0.07141
207	-16.356	422.786	116.904	-1.636	1	0.00042	-0.08031
208	-17.601	415.645	116.904	-1.760	1	0.00042	-0.08642
209	-17.931	409.145	116.904	-1.793	1	0.00042	-0.08804
210	-17.628	403.201	116.904	-1.763	1	0.00042	-0.08656
211	-17.224	397.863	116.904	-1.722	1	0.00042	-0.08457
212	-16.803	393.018	116.904	-1.680	1	0.00042	-0.08250
213	-16.672	388.659	116.904	-1.667	1	0.00042	-0.08186
214	-16.744	384.734	116.904	-1.674	1	0.00042	-0.08221
215	-16.827	381.076	116.904	-1.683	1	0.00042	-0.08262
216	-16.977	377.620	116.904	-1.698	1	0.00042	-0.08336
217	-17.070	374.350	116.904	-1.707	1	0.00042	-0.08381



Wavelength (nm)	CD (θ)	HT	MRW	$\theta/10$	d (cm.)	c (g/mL)	$[\theta]$ MRE, λ
218	-17.119	371.221	116.904	-1.712	1	0.00042	-0.08405
219	-17.165	368.098	116.904	-1.717	1	0.00042	-0.08428
220	-17.159	365.020	116.904	-1.716	1	0.00042	-0.08425
221	-17.186	362.063	116.904	-1.719	1	0.00042	-0.08438
222	-17.260	359.150	116.904	-1.726	1	0.00042	-0.08475
223	-17.197	356.246	116.904	-1.720	1	0.00042	-0.08444
224	-16.919	353.331	116.904	-1.692	1	0.00042	-0.08307
225	-16.356	350.338	116.904	-1.636	1	0.00042	-0.08031
226	-15.526	347.250	116.904	-1.553	1	0.00042	-0.07623
227	-14.644	344.059	116.904	-1.464	1	0.00042	-0.07190
228	-13.746	340.772	116.904	-1.375	1	0.00042	-0.06749
229	-12.715	337.446	116.904	-1.271	1	0.00042	-0.06243
230	-11.693	334.118	116.904	-1.169	1	0.00042	-0.05741
231	-10.608	330.773	116.904	-1.061	1	0.00042	-0.05209
232	-9.361	327.366	116.904	-0.936	1	0.00042	-0.04596
233	-8.163	323.923	116.904	-0.816	1	0.00042	-0.04008
234	-6.992	320.548	116.904	-0.699	1	0.00042	-0.03433
235	-5.845	317.290	116.904	-0.584	1	0.00042	-0.02870
236	-4.930	314.198	116.904	-0.493	1	0.00042	-0.02420
237	-4.188	311.287	116.904	-0.419	1	0.00042	-0.02056
238	-3.541	308.592	116.904	-0.354	1	0.00042	-0.01739
239	-2.980	306.155	116.904	-0.298	1	0.00042	-0.01463
240	-2.398	303.950	116.904	-0.240	1	0.00042	-0.01178
241	-1.845	301.966	116.904	-0.185	1	0.00042	-0.00906
242	-1.353	300.154	116.904	-0.135	1	0.00042	-0.00664
243	-1.011	298.489	116.904	-0.101	1	0.00042	-0.00496
244	-0.858	296.945	116.904	-0.086	1	0.00042	-0.00422
245	-0.754	295.502	116.904	-0.075	1	0.00042	-0.00370
246	-0.643	294.164	116.904	-0.064	1	0.00042	-0.00316
247	-0.518	292.898	116.904	-0.052	1	0.00042	-0.00254
248	-0.347	291.743	116.904	-0.035	1	0.00042	-0.00170
249	-0.207	290.720	116.904	-0.021	1	0.00042	-0.00102
250	-0.165	289.740	116.904	-0.016	1	0.00042	-0.00081
251	-0.148	288.812	116.904	-0.015	1	0.00042	-0.00073
252	-0.188	287.933	116.904	-0.019	1	0.00042	-0.00092
253	-0.197	287.073	116.904	-0.020	1	0.00042	-0.00097
254	-0.129	286.224	116.904	-0.013	1	0.00042	-0.00063
255	-0.033	285.371	116.904	-0.003	1	0.00042	-0.00016
256	0.024	284.498	116.904	0.002	1	0.00042	0.00012
257	0.047	283.611	116.904	0.005	1	0.00042	0.00023



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Wave length (nm)	CD (θ)	HT	MRW	$\theta/10$	d (cm.)	c (g/ml)	$[\theta]$ MRE, λ
258	0.056	282.704	116.904	0.006	1	0.00042	0.00028
259	0.075	281.734	116.904	0.007	1	0.00042	0.00037
260	0.034	280.725	116.904	0.003	1	0.00042	0.00017
261	-0.029	279.811	116.904	-0.003	1	0.00042	-0.00014
262	-0.112	278.981	116.904	-0.011	1	0.00042	-0.00055
263	-0.136	278.201	116.904	-0.014	1	0.00042	-0.00067
264	-0.134	277.433	116.904	-0.013	1	0.00042	-0.00066
265	-0.115	276.692	116.904	-0.012	1	0.00042	-0.00057
266	-0.142	275.995	116.904	-0.014	1	0.00042	-0.00070
267	-0.168	275.335	116.904	-0.017	1	0.00042	-0.00082
268	-0.223	274.705	116.904	-0.022	1	0.00042	-0.00109
269	-0.260	274.081	116.904	-0.026	1	0.00042	-0.00128
270	-0.291	273.427	116.904	-0.029	1	0.00042	-0.00143
271	-0.325	272.716	116.904	-0.033	1	0.00042	-0.00160
272	-0.313	272.028	116.904	-0.031	1	0.00042	-0.00154
273	-0.263	271.434	116.904	-0.026	1	0.00042	-0.00129
274	-0.187	270.858	116.904	-0.019	1	0.00042	-0.00092
275	-0.169	270.278	116.904	-0.017	1	0.00042	-0.00083
276	-0.179	269.674	116.904	-0.018	1	0.00042	-0.00088
277	-0.221	269.070	116.904	-0.022	1	0.00042	-0.00108
278	-0.263	268.487	116.904	-0.026	1	0.00042	-0.00129
279	-0.343	267.907	116.904	-0.034	1	0.00042	-0.00168
280	-0.370	267.395	116.904	-0.037	1	0.00042	-0.00182
281	-0.371	267.025	116.904	-0.037	1	0.00042	-0.00182
282	-0.333	266.743	116.904	-0.033	1	0.00042	-0.00163
283	-0.240	266.476	116.904	-0.024	1	0.00042	-0.00118
284	-0.194	266.195	116.904	-0.019	1	0.00042	-0.00095
285	-0.245	265.896	116.904	-0.025	1	0.00042	-0.00120
286	-0.288	265.566	116.904	-0.029	1	0.00042	-0.00141
287	-0.297	265.252	116.904	-0.030	1	0.00042	-0.00146
288	-0.279	264.987	116.904	-0.028	1	0.00042	-0.00137
289	-0.213	264.682	116.904	-0.021	1	0.00042	-0.00105
290	-0.200	264.172	116.904	-0.020	1	0.00042	-0.00098
291	-0.216	263.690	116.904	-0.022	1	0.00042	-0.00106
292	-0.245	263.552	116.904	-0.024	1	0.00042	-0.00120
293	-0.290	263.455	116.904	-0.029	1	0.00042	-0.00142
294	-0.296	263.176	116.904	-0.030	1	0.00042	-0.00145
295	-0.276	262.828	116.904	-0.028	1	0.00042	-0.00136
296	-0.281	262.479	116.904	-0.028	1	0.00042	-0.00138
297	-0.247	262.123	116.904	-0.025	1	0.00042	-0.00121



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Wave.length (nm)	CD (θ)	HT	MRW	$\theta/10$	d (cm.)	c (g/ml)	$[\theta]$ MRE, λ
298	-0.217	261.763	116.904	-0.022	1	0.00042	-0.00107
299	-0.209	261.417	116.904	-0.021	1	0.00042	-0.00103
300	-0.196	261.106	116.904	-0.020	1	0.00042	-0.00096
301	-0.205	260.824	116.904	-0.020	1	0.00042	-0.00101
302	-0.218	260.573	116.904	-0.022	1	0.00042	-0.00107
303	-0.188	260.342	116.904	-0.019	1	0.00042	-0.00092
304	-0.132	260.124	116.904	-0.013	1	0.00042	-0.00065
305	-0.058	259.915	116.904	-0.006	1	0.00042	-0.00029
306	-0.041	259.728	116.904	-0.004	1	0.00042	-0.00020
307	-0.081	259.571	116.904	-0.008	1	0.00042	-0.00040
308	-0.134	259.435	116.904	-0.013	1	0.00042	-0.00066
309	-0.194	259.306	116.904	-0.019	1	0.00042	-0.00095
310	-0.216	259.175	116.904	-0.022	1	0.00042	-0.00106
311	-0.179	258.978	116.904	-0.018	1	0.00042	-0.00088
312	-0.161	258.741	116.904	-0.016	1	0.00042	-0.00079
313	-0.157	258.650	116.904	-0.016	1	0.00042	-0.00077
314	-0.152	258.707	116.904	-0.015	1	0.00042	-0.00075
315	-0.187	258.786	116.904	-0.019	1	0.00042	-0.00092
316	-0.222	258.807	116.904	-0.022	1	0.00042	-0.00109
317	-0.231	258.794	116.904	-0.023	1	0.00042	-0.00113
318	-0.221	258.777	116.904	-0.022	1	0.00042	-0.00109
319	-0.170	258.746	116.904	-0.017	1	0.00042	-0.00083
320	-0.090	258.701	116.904	-0.009	1	0.00042	-0.00044



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PRESENTATIONS

1. The 21st Science Forum 2013. March 14-15, 2013. Faculty of Science, Chulalongkorn University, Bangkok, Thailand. “Characterization of viral responsive protein 15 and its role in cell import/export of virus in black tiger shrimp *Penaeus monodon*” (Poster presentation)
2. The 18th Biological Sciences Graduate Congress. January 6-8, 2014. Faculty of Science, University of Malaya, Kuala Lumpur, Malaysia. “Characterization of viral responsive protein 15 and its role in cell import/export of virus in black tiger shrimp *Penaeus monodon*” (Poster presentation)

Award : The Bronze Medal Winner for poster presentation award in theme: Cell and Molecular Biology of the 18th Biological Sciences Graduate Congress at University of Malaya, Kuala Lumpur, Malaysia.



VITA

Miss Krisadaporn Jaturontakul was born on June 22, 1989 in Bangkok. She graduated with the degree of Bachelor of Science from the Department of Biochemistry, Faculty of Science, Chulalongkorn University in 2010. She has studied for the Master's degree of Science at the Department of Biochemistry, Faculty of Science, Chulalongkorn University since 2011. In the third year of her research work, she presented a poster entitle "Cloning and expression of Viral Responsive Protein 15 from black tiger shrimp *Penaeus monodon*" at NGRC 29th The National Graduate Research Conference, organized by Mae Fah Luang University on October 24-25, 2013.

