

Inhibit *Procambarus clarkii* from
the infection of WSSV by WSSV
reconstructed viral attachment
protein 1 (rVAP1)

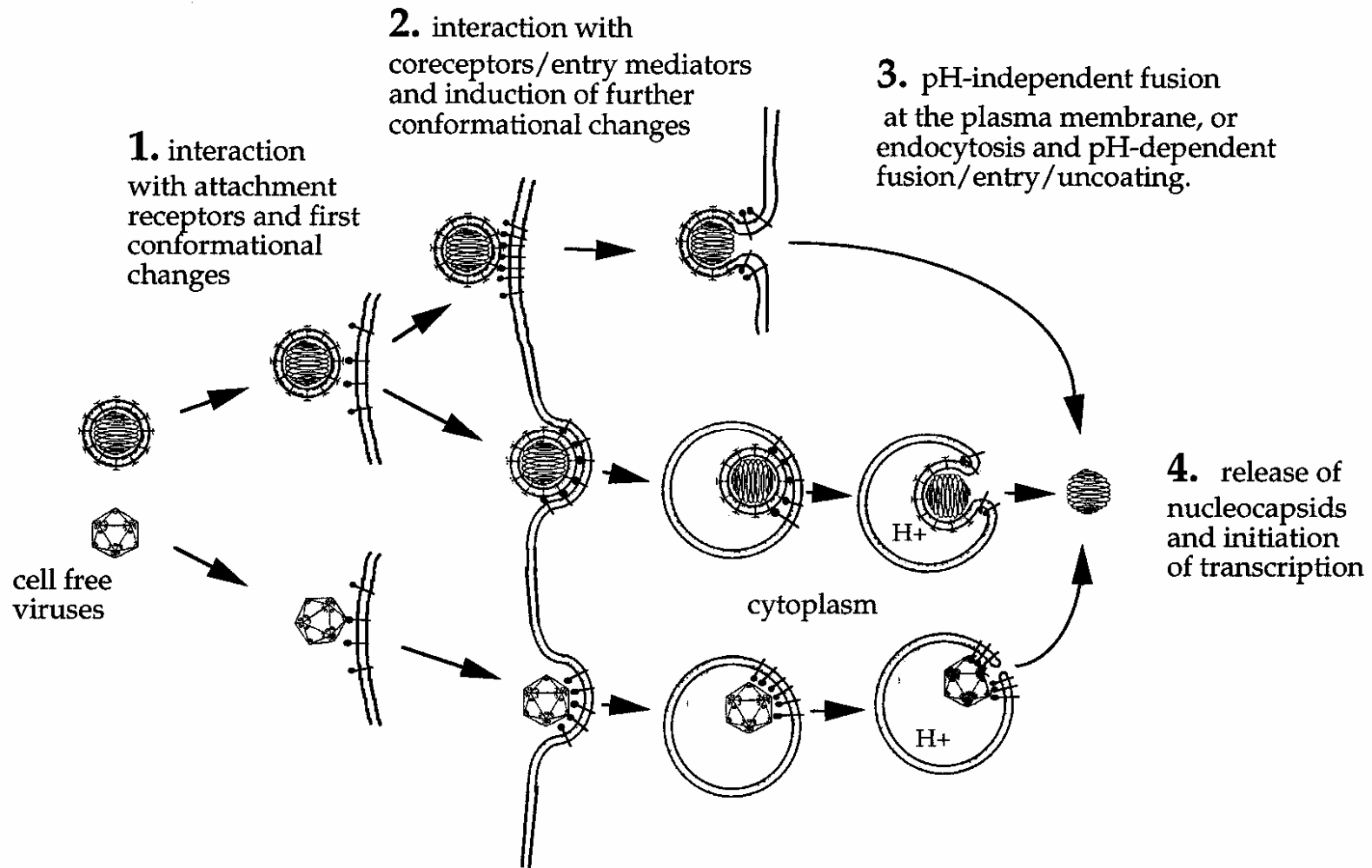
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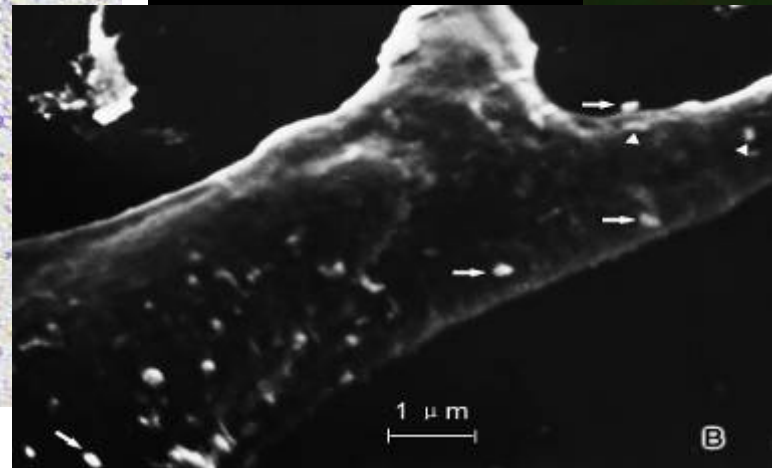
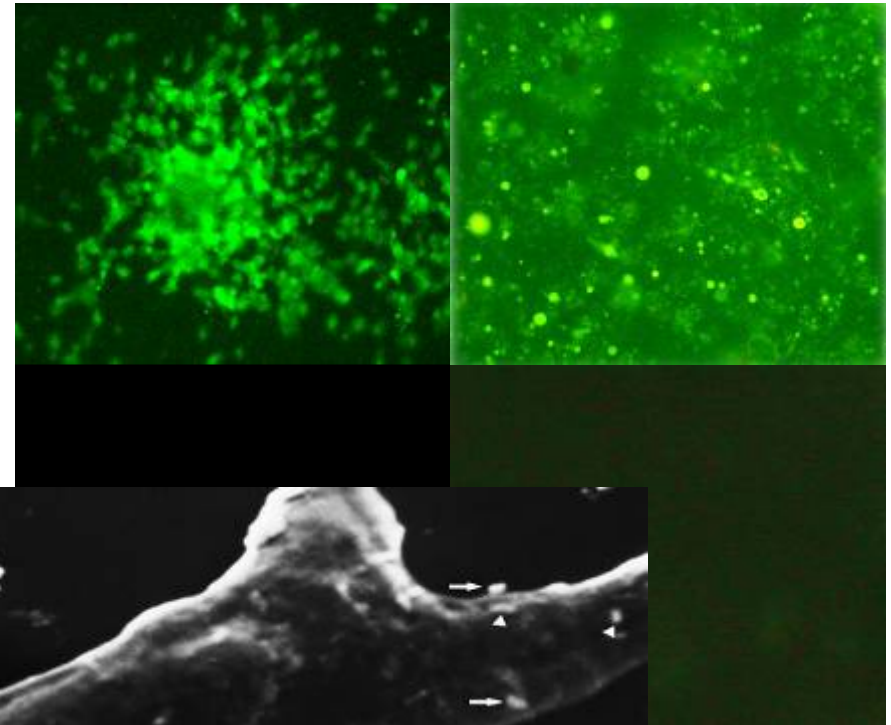
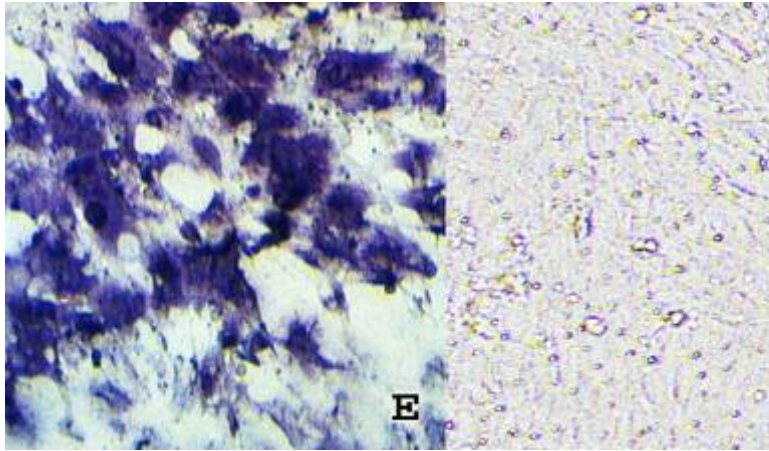
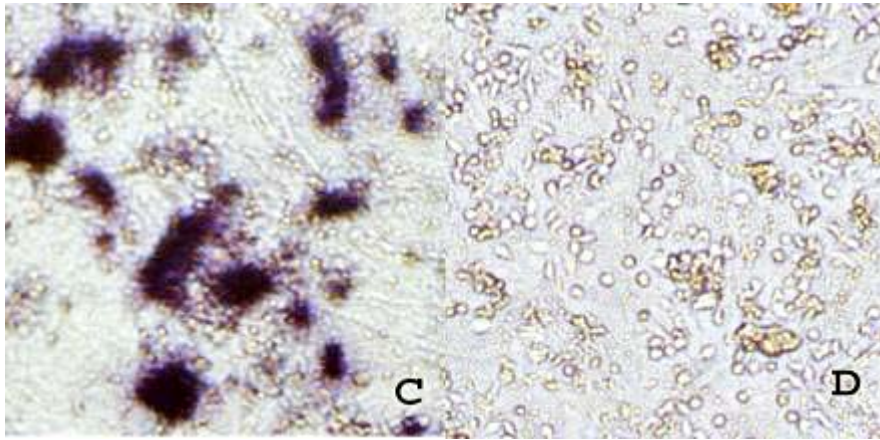
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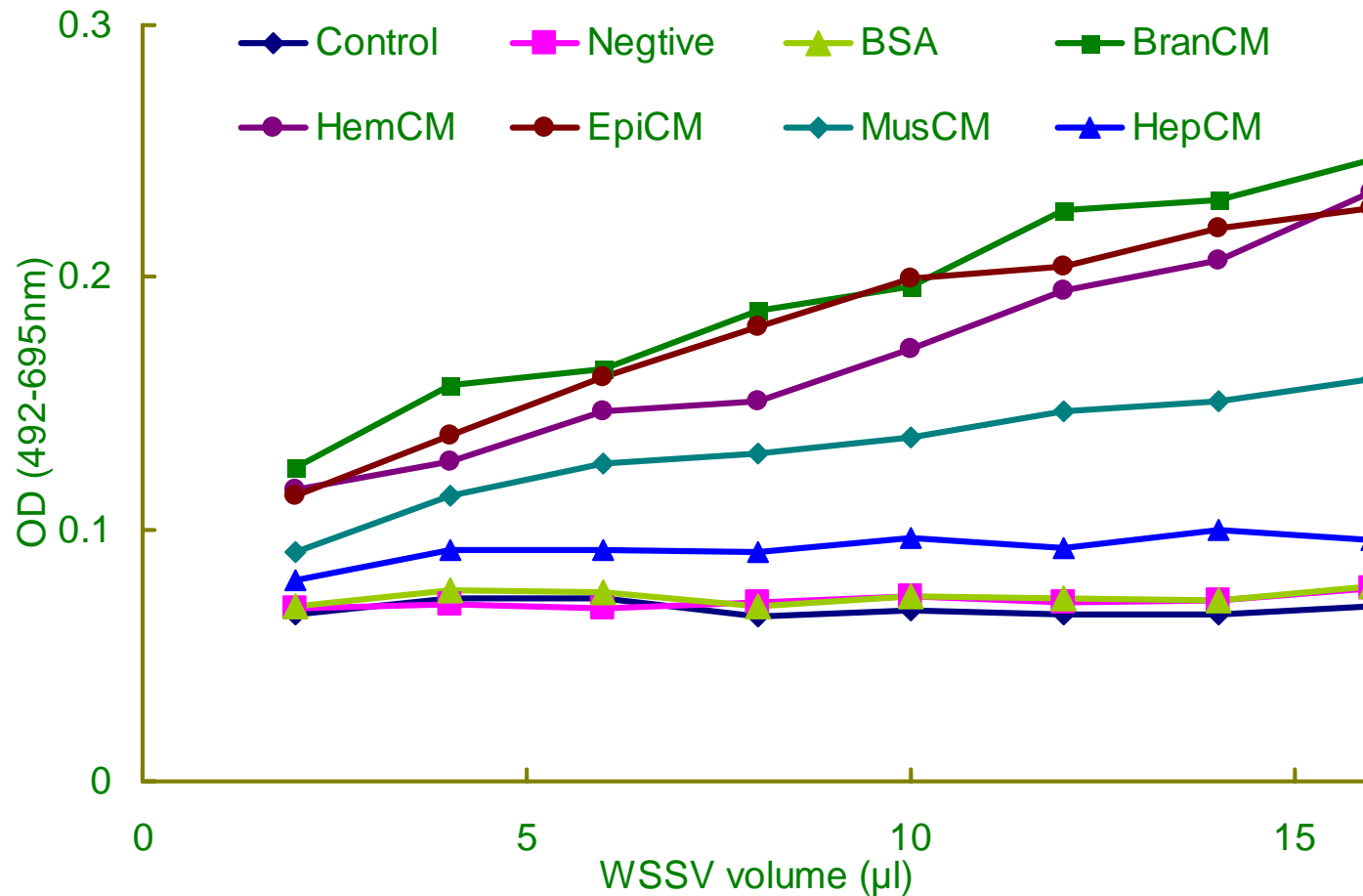
Steps of Cell Entry by Virus



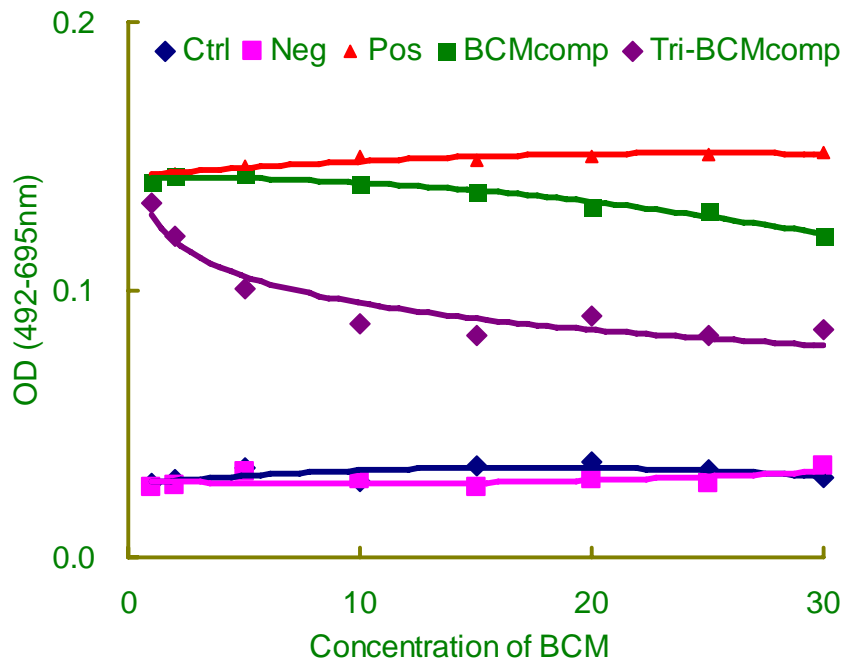
Binding activity of WSSV to host cells



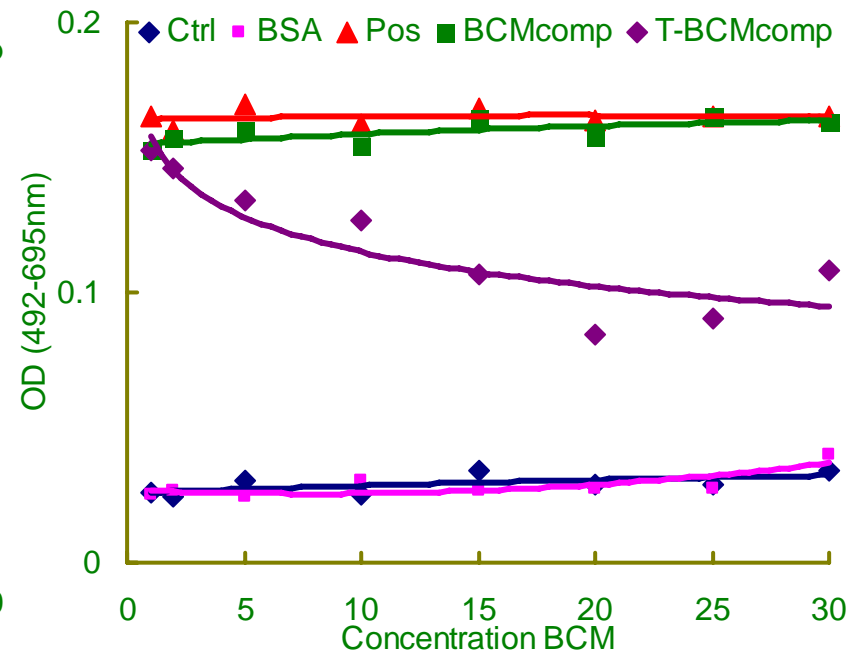
Binding to different cell membranes



Competitive inhibition of binding activity between different cellular membranes



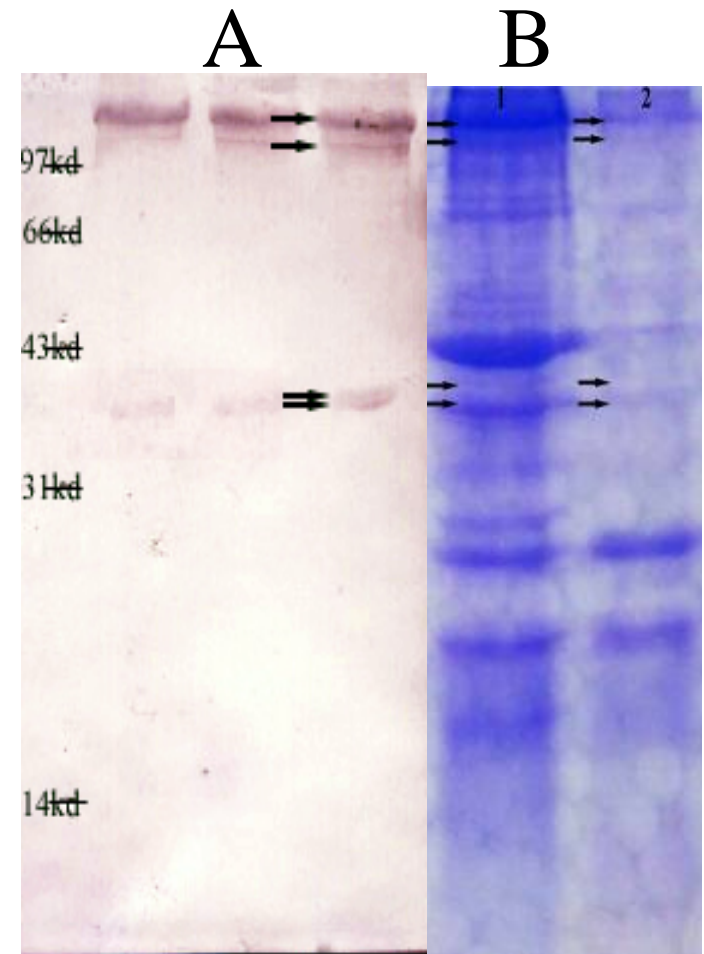
Binding to hemocyte membrane of *P. vannamei*



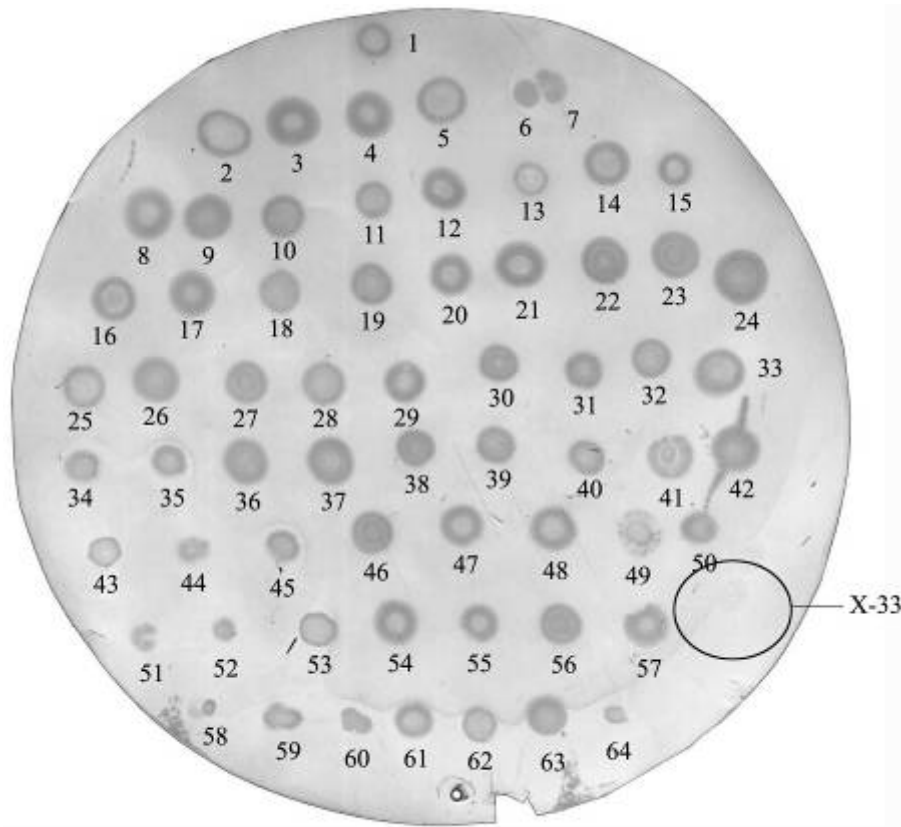
Binding to Epithelium cell membrane of *P. vannamei*

Viral Outlay Proteins Binding Assay (VOPBA) by Western Blot

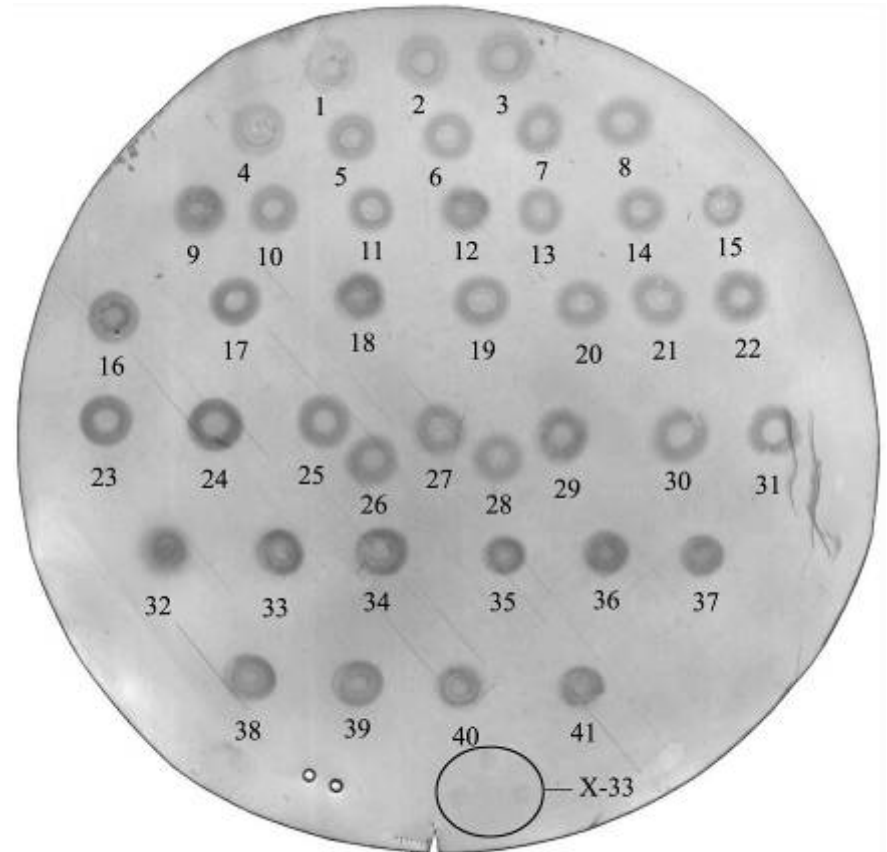
- Binding activity of WSSV is resistant to SDS treatment.
- SDS-PAGE of WSSV was blotted to cellulose nitrate membrane (CNM). After blocking, DIG labeled cellular membrane component binds with the CNM at 4 ° C overnight. Then the CNM was develop.
- Result show two regions on the CNM.
 - The first region is above 97.4 Kd, including 2 bands.
 - The second region is between 43 Kd and 30 Kd, including 2 bands.



Recombination and expression of VAP1 by *Pichia pastoris* X-33



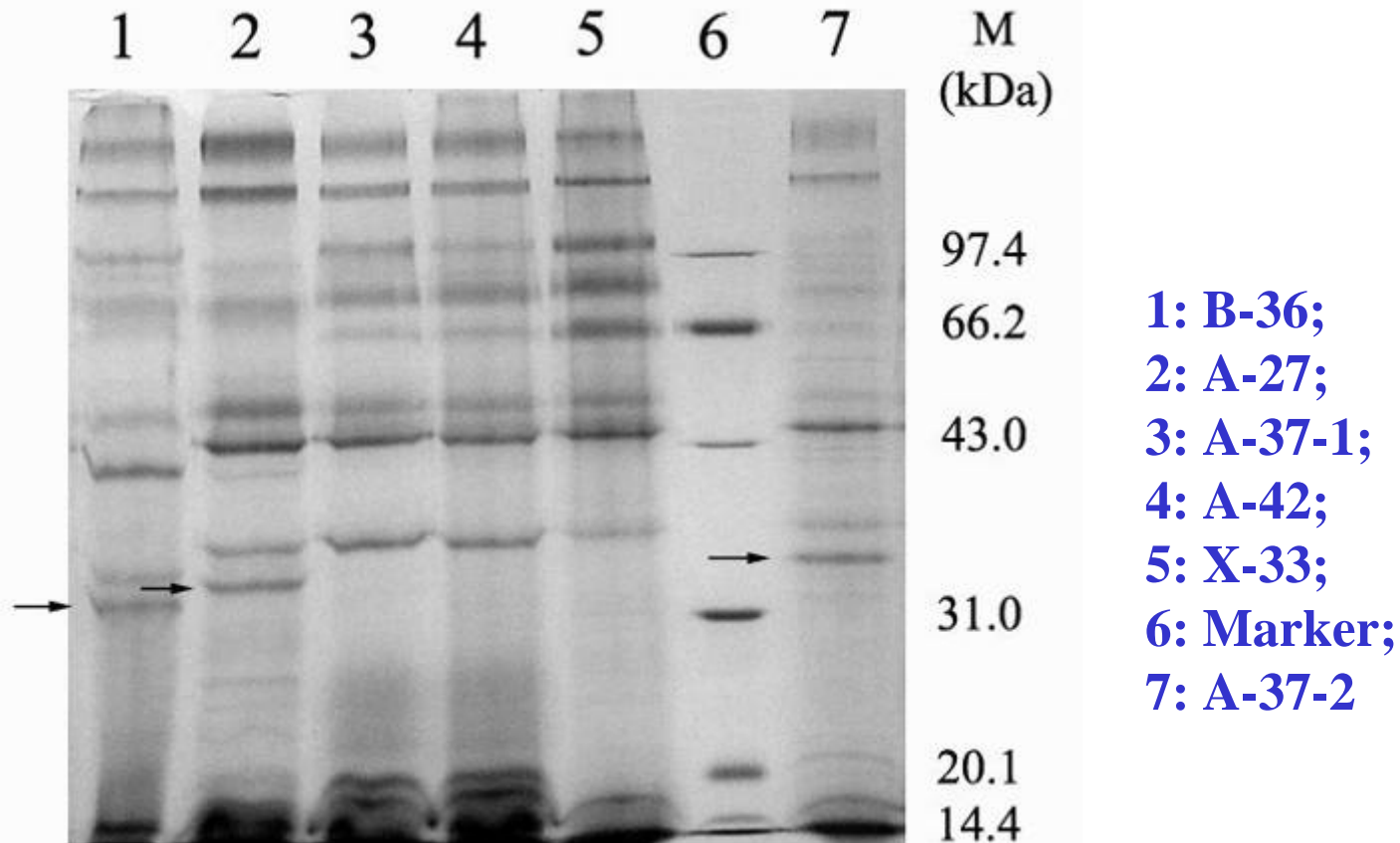
A



B

The blotting membrane for selecting high-level expressing transformers

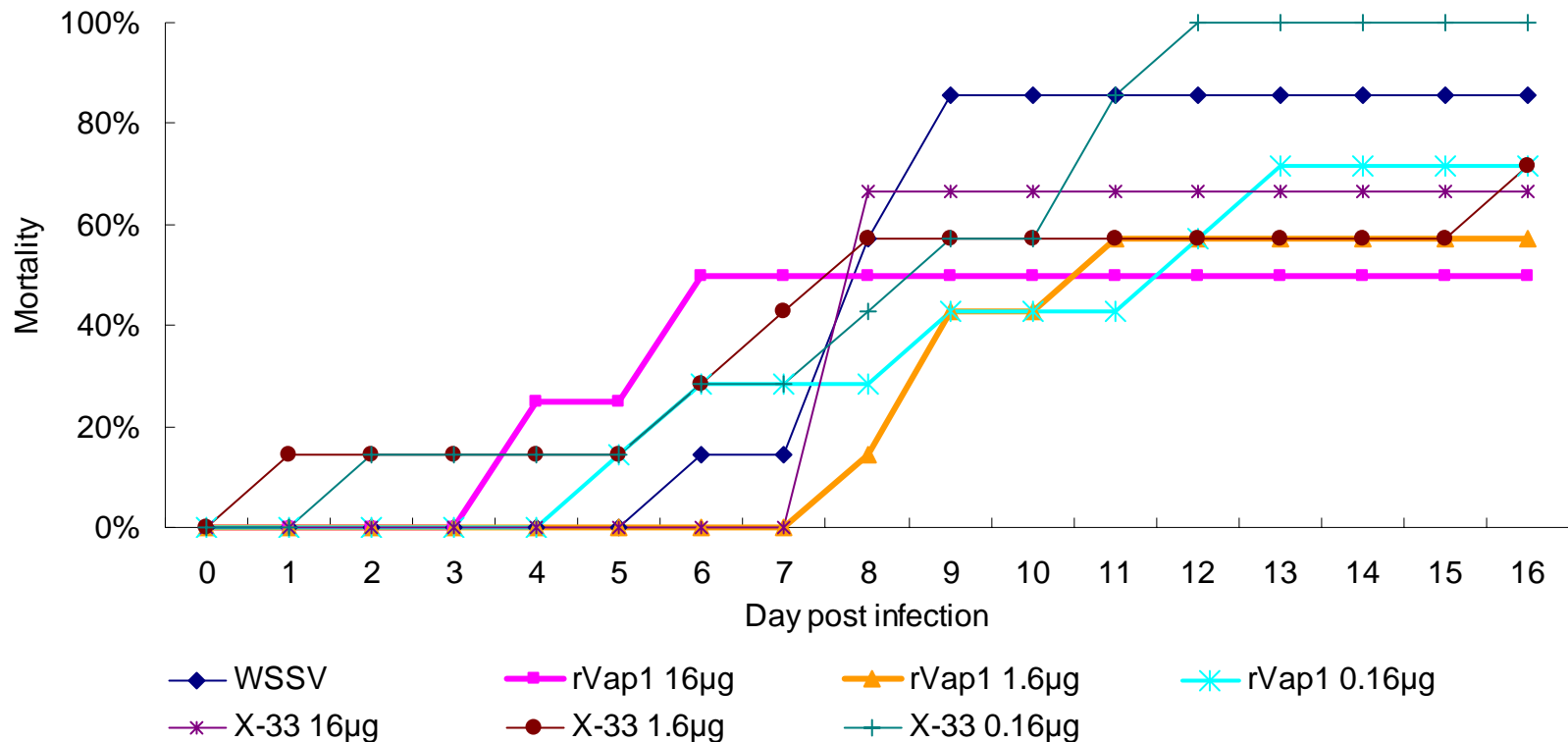
SDS-PAGE analysis for selecting high expression clones



The binding capability of rVAP1 to shrimp membrane proteins

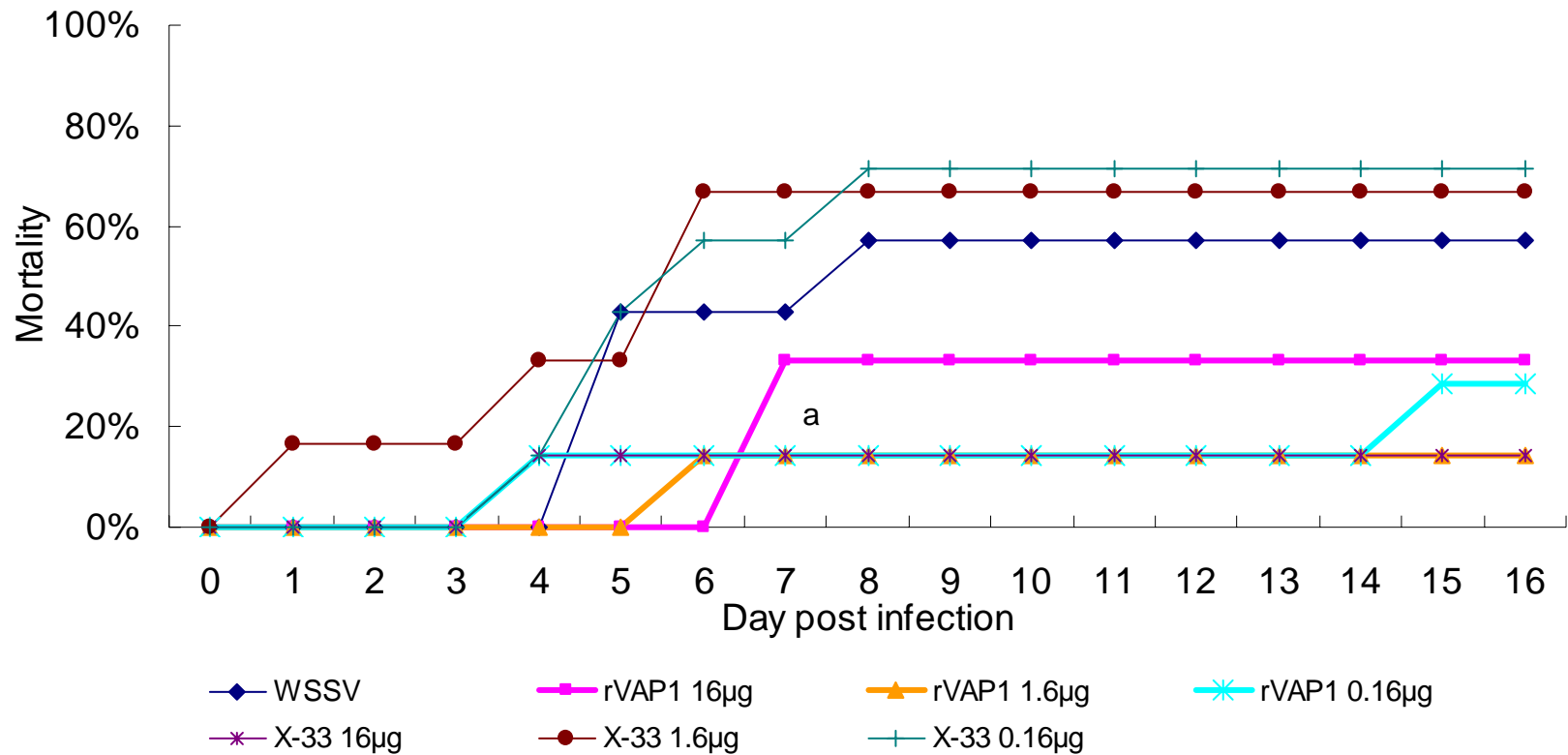
Cellular membrane	Branchial cells	Hemocytes	Heart	Lymphoid organ
A-27(P)	1.24	1.89	1.98	1.47
X-33(N)	0.12	0.23	0.25	0.28
P/N	10.33	8.22	7.92	5.25

Protection of rVAP1 from WSSV infection



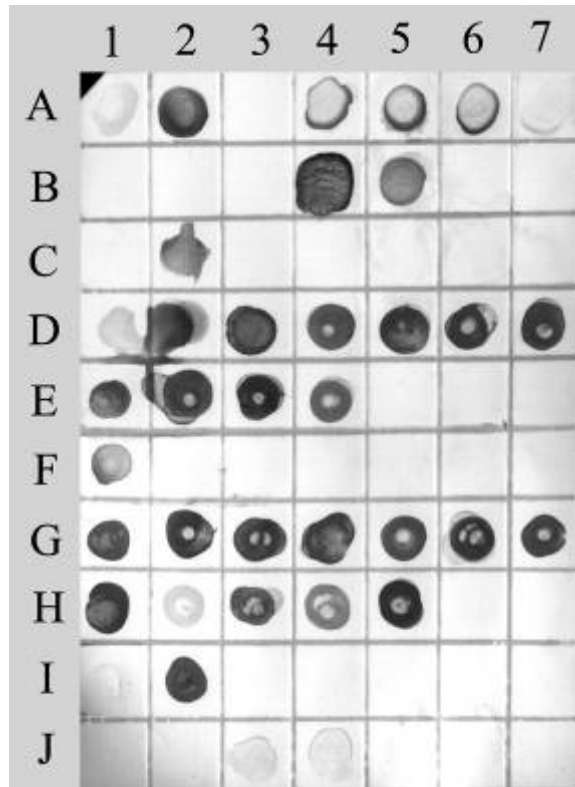
Infection with 6.81 LD₅₀ WSSV

Protection of rVAP1 from WSSV infection

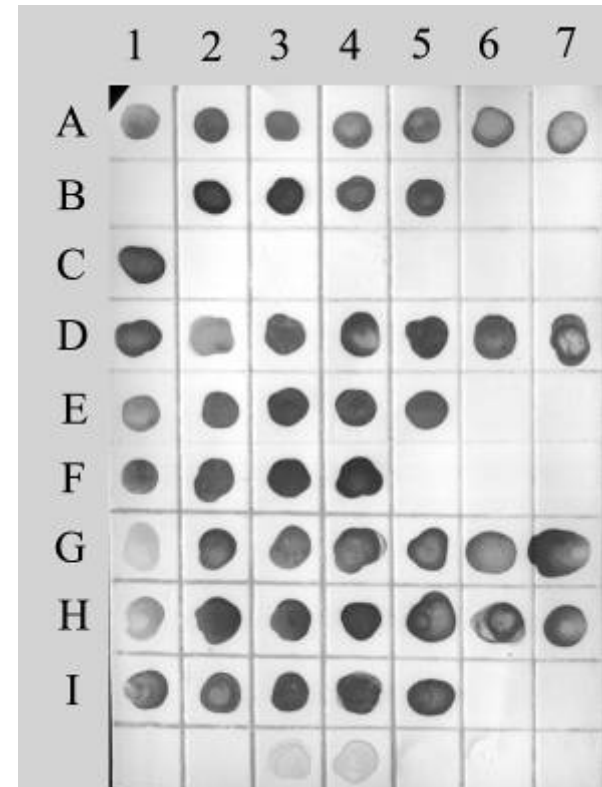


Infection with 0.681 LD₅₀ WSSV

Demonstration of WSSV infection by DNA probe



Injection with rVAP1



Injection with Extracts of X-33

Protection index of rVAP1

Groups	Final LD ₅₀	Protection index
rVAP1 16μg	7.28 x 10 ⁻⁵ g/ml	6.28
rVAP1 1.6μg	5.78 x 10 ⁻⁵ g/ml	4.78
rVAP1 0.16μg	3.16 x 10 ⁻⁵ g/ml	2.16
X-33 16μg	4.50 x 10 ⁻⁵ g/ml	3.50
X-33 1.6μg	1.15x 10 ⁻⁵ g/ml	0.15
X-33 0.16μg	-	-
WSSV control	1 x 10 ⁻⁵ g/ml	0



Great Thanks!