Supplementary Information for:

In vitro template-change PCR to create single crossover libraries: a case study with *B. thuringiensis* Cry2A toxins.

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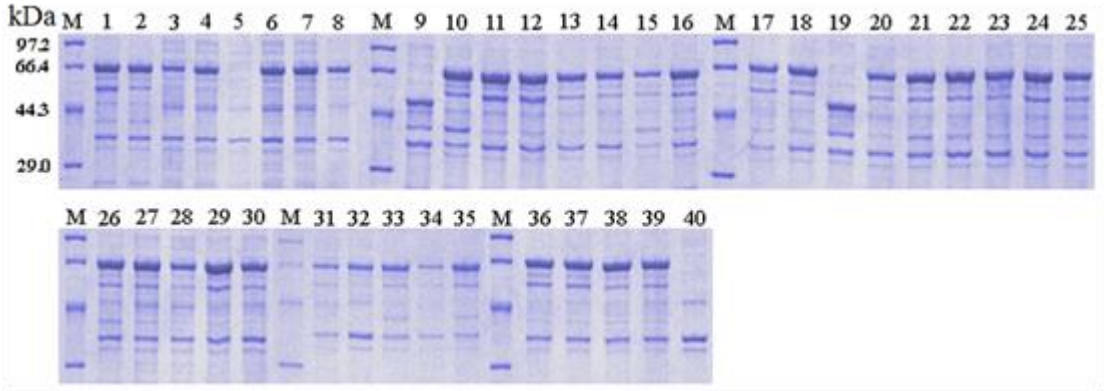
Supplementary Figure 1. SDS PAGE analysis of the expressed toxins.



Expression of *cry2Aa*, *cry2Ad* and recombinants in *E. coli* Rosetta. M： Low MW marker; Lane 1: Cry2Aa; Lane 2: Cry2Ad; Lane 40: pEB control; Lanes 3-39: chimeras R01-R37.

Supplementary Table 1. Sequences of primers used in this study.

Name	Sequence
F	5′-ATGAATAATGTATTGAATAGTGGA-3′
R	5′-ATAAAGTGGTGGAAGATTAGTT-3′
F′	5′-ATGAATAGTGTATTGAATAGCGGA-3′
LR	5′-TTAGCTGGAAACGCGTCGACATAAAGTGGTGAAAGATT-3′
LF	5′-AACCTTGTAGCGCGGATCCGATGAATAATGTATTGAAT-3′
SR	5′-TTAGCTGGAAACGCGTCGAC-3′
SF	5′-AACCTTGTAGCGCGGATCCG-3′
P1	5′-ATGAATAATGTATTGAATAGTGG-3′
P1′	5′-CAGGAGCTACATATAAACTATGAT-3′
P2	5′-TGATAACGGAGCTCGTTT-3′
P2′	5′-ATAAAGTGGTGAAAGATTAGTTGG-3′

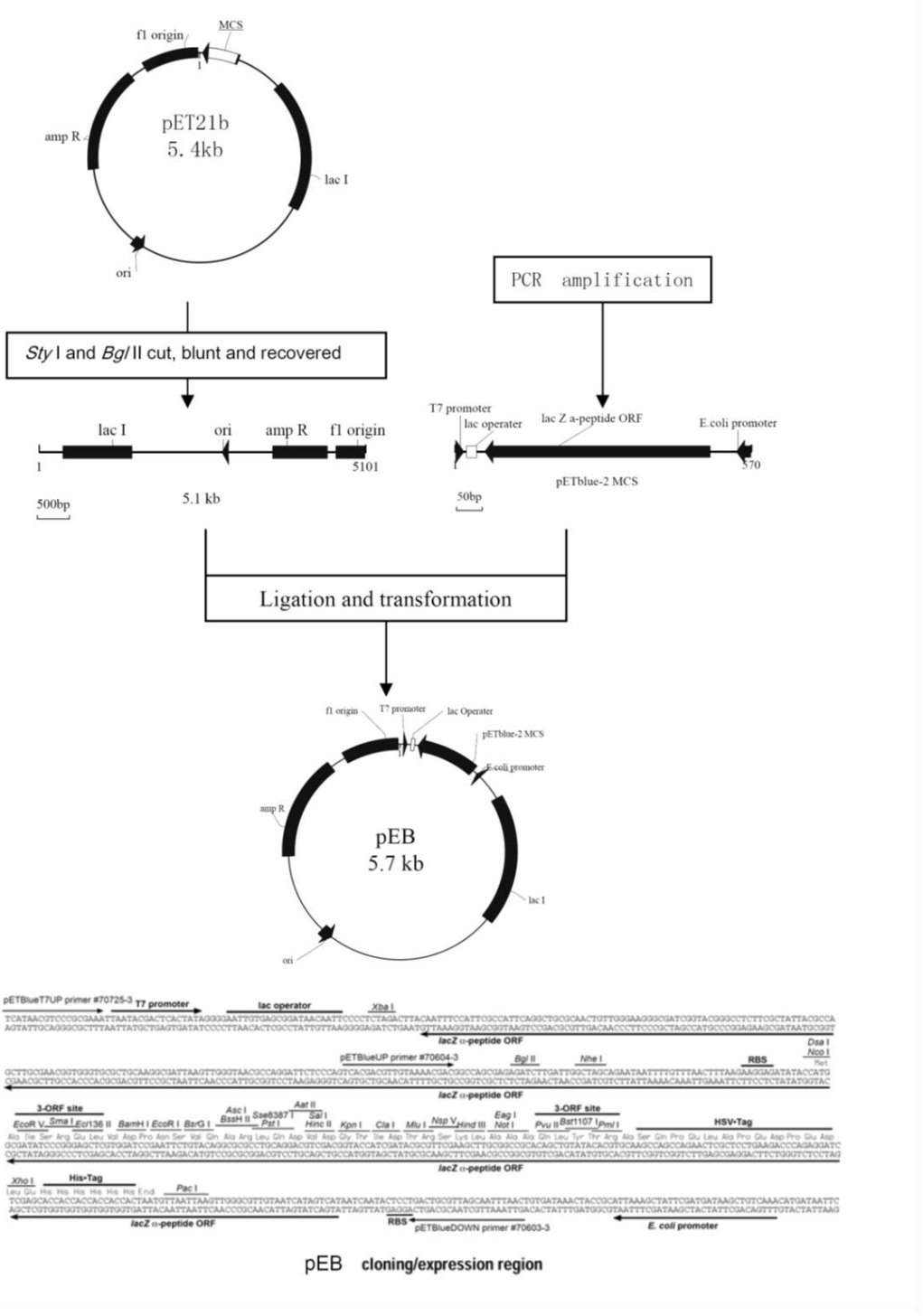
Supplementary Table 2 Bioassay of the toxicities of the chimeras and parent proteins.

Samples [a]	*O. furnacalis*		*P. xylostella*		*C. suppressalis*		*H. armigera*	
	Mortality	**SD**	**Mortality**	**SD**	**Mortality**	**SD**	**Mortality**	**SD**
Control	0.00	0.00	0.00	0.58	6.67	0.58	2.22	0.58
Cry2Ad	0.00	0.00	4.55	0.00	3.45	1.53	9.09	1.53
R01	0.00	0.00	15.56	2.08	3.33	1.15	4.55	1.73
R02	0.00	0.00	6.67	1.73	3.33	1.15	2.27	0.58
R04	5.00	1.00	4.44	1.15	6.67	1.53	9.09	2.08
R05	0.00	0.00	6.67	1.00	13.33	1.53	9.09	1.15
R06	0.00	0.00	8.89	0.58	6.67	0.58	11.36	1.00
R08	0.00	0.00	13.33	1.00	16.67	1.53	2.27	0.58
R09	1.67	0.58	11.11	1.15	10.00	1.73	13.64	1.53
R10	1.67	0.58	11.11	1.53	6.67	1.53	4.55	1.73
R11	3.33	0.58	15.56	1.15	16.67	1.53	11.36	1.00
R12	1.67	0.58	4.44	1.15	13.33	0.58	6.82	1.15
R13	0.00	0.00	6.67	1.73	6.67	2.31	13.64	0.58
R14	0.00	0.00	8.89	1.15	10.00	2.00	13.64	1.15
R15	0.00	0.00	8.89	0.58	10.00	1.73	9.09	1.53
R16	5.00	1.00	2.22	0.58	6.67	0.58	11.36	1.73
R18	0.00	0.00	15.56	2.08	10.00	1.00	6.82	0.58
R19	3.33	1.15	4.44	1.15	6.67	2.31	9.09	2.08
R20	3.33	1.15	2.22	0.58	6.67	1.53	11.36	1.00
R21	5.00	1.73	8.89	1.15	8.33	1.53	6.82	1.15
R22	1.67	0.58	13.33	0.00	10.00	2.65	15.91	1.15

R23	5.00	1.00	15.56	2.08	11.67	2.52	9.09	1.15
R24	**26.67**	1.15	15.56	1.15	13.33	1.15	9.09	2.08
R25	**36.67**	0.58	13.33	1.73	13.33	0.58	13.64	0.58
R26	**36.67**	1.53	**75.56**	0.58	16.67	2.52	20.45	2.52
R27	**38.33**	1.15	**80.00**	0.00	**76.67**	1.53	**84.09**	1.15
R28	**28.33**	3.06	**82.22**	0.58	**76.67**	0.58	**86.36**	1.00
R29	**28.33**	0.58	**77.78**	0.58	**80.00**	1.00	**93.18**	1.00
R30	**38.33**	1.15	**82.22**	1.15	**75.00**	0.00	**90.91**	1.53
R31	**33.33**	1.53	**86.67**	0.00	**73.33**	0.58	**81.82**	1.53
R32	**33.33**	3.06	**84.44**	0.58	**76.67**	0.58	**93.18**	1.73
R33	**36.67**	1.15	**82.22**	0.58	**80.00**	1.00	**86.36**	1.00
R34	**30.00**	2.00	**75.56**	0.58	**78.33**	1.53	**84.09**	0.58
R35	**31.67**	1.15	**80.00**	0.00	**73.33**	1.53	**84.09**	2.08
R36	**36.67**	3.06	**88.89**	1.53	**80.00**	0.00	**88.64**	0.58
R37	**35.00**	1.73	**82.22**	0.58	**76.67**	0.58	**86.36**	1.00
Cry2Aa	**33.33**	1.15	**82.22**	0.58	**76.67**	0.58	**97.73**	0.58

[a] R01 through R37 are chimeras produced for this study. The crossover point for each chimera is shown in Figure 2. The control assay was performed using sample buffer alone. The data show percentage larval mortalities (average of three independent experiments) after exposure to the indicated proteins (50 ppm), mortalities shown in bold refer to activities that were statistically comparable to that observed with Cry2Aa. SD means standard deviation.

Supplementary Figure 1



pEB cloning/expression region

Supplementary Fig. 1: Construction of the expression vector. pEB. This was constructed by replacing the expression region of pET21b with that of pETblue-2. The resulting plasmid contains the *lacI* gene and places the T7 promoter directly under the control of the *lac* operator.