

Preparation and Purification of Porcine Circovirus Type 2 Chimeric Virus-Like Particles by a New Affinity Purification System

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Abstract

Porcine epidemic diarrhea virus (PEDV) causes severe watery diarrhea with dehydration and high mortality in pigs, bringing huge economic losses to the swine industry worldwide. The current prevention of PEDV is mainly concentrated on inactivated vaccines. In this study, two novel B cell epitopes S1D5 (residues 744-759) and S1D6 (residues 756-771) of PEDV were displayed on capsid (Cap) protein of porcine circovirus type 2 (PCV2) in *Escherichia coli* (*E. coli*) expression system. The recombinant protein Cap-S1D can self-assemble into chimeric virus-like particles (VLPs), which were similar in size and morphology to native PCV2 particles. In addition, an effective affinity purification system for the purification of chimeric VLPs, referred to Gram-positive Enhancer Matrix-protein anchor domain-variable domain of heavy chain of heavy-chain antibody 8 (GEM-PA-VHH8), was screened and identified. The PA-VHH8 fusion protein can specifically bind to GEM particles by covalent bond adsorption, which contributes to display Cap-S1D chimeric VLPs on the surface of GEM particles. Cap-S1D chimeric VLPs were successfully purified with the developed purification system and the purification efficiency was close to 90%. What's more, the purified chimeric VLPs homemade vaccine induced a stronger humoral and cellular immune responses compared to the unpurified recombinant protein in pigs. Thus, the GEM-PA-VHH system provided an effective and safe technology for the preparation and purification of chimeric VLPs to prevent PEDV and PCV2 infection.

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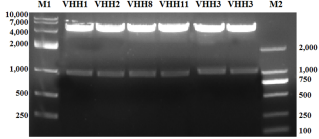


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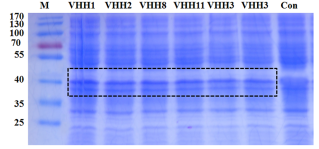
	<i>FR1(A41-29)</i>	<i>CDR1(A436-35)</i>	<i>FR2(A436-49)</i>	<i>CDR3(A458-79)</i>
VHH1	DVQVSGGGVGGAGGSLRLCTAS	GFTFDOTMG	WFRQAPGKEREVS	YTWGSGMSGSTYYTDSVKG
VHH2	DVQVSGGGVGGAGGSLRLCTAS	GFTFDOTMG	WFRQAPGKEREVS	YTWGSGTSGSTYYTDSVKG
VHH3	DVQVSGGGVGGAGGSLRLCTAS	GFTYSNHLG	WFRQTPGKEREVA	AYWGLRGGRTYADSVKG
VHH8	DVQVSGGGVGGAGGSLRLCAGS	GWTMSKMG	WFRQAPGKEREVA	RYTDGSPFYADSVKG
VHH11	DVQVSGGGVGGAGGSLRLCTAS	GFTFDCTMG	WFRQAPGKEREVS	YTWGSGTSGSTYYTDSVKG

	<i>FR3(A471-102)</i>	<i>CDR3(A4103-119)</i>	<i>CDR2(A4128-132)</i>
VHH1	RFTISRDNAENTYLGINSFKPEDTAMYCAA	ARYRGSGQVRWTDHY	WGQGTQTVYS
VHH2	RFTISRDNAENTYLGINSFKPEDTAMYCAA	ARYRGSGQVRWTDHY	WGQGTQTVYS
VHH3	RFTISRDNAENTYLGINSFKPEDTAMYCAA	STRINAGVDWYRPFNY	WGQGTQTVYS
VHH8	RFTISRDNAENTYLGINSFKPEDTAMYCAA	SKRGRSGWVPKASSTYFDG	WGQGTQTVYS
VHH11	RFTISRDNAENTYLGINSFKPEDTAMYCAA	ARYRGSGQVRWTDHY	WGQGTQTVYS

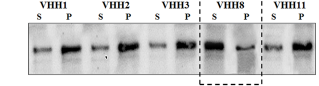
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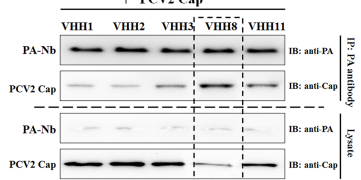
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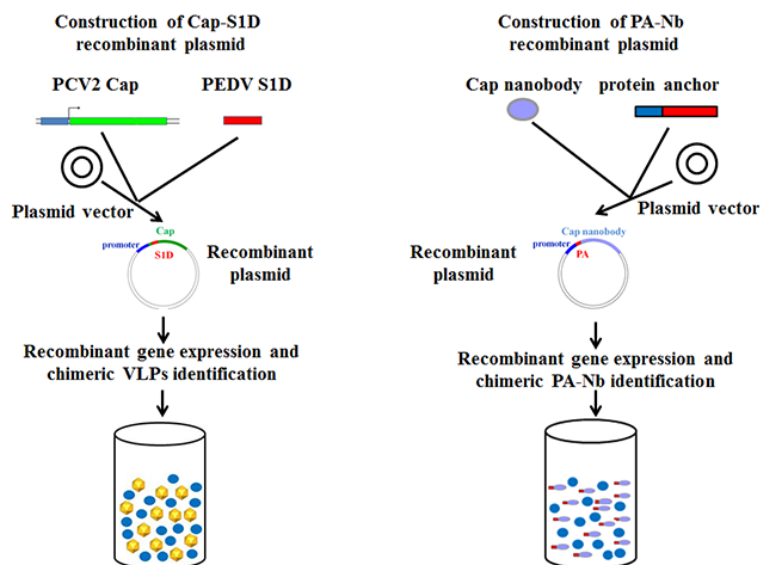
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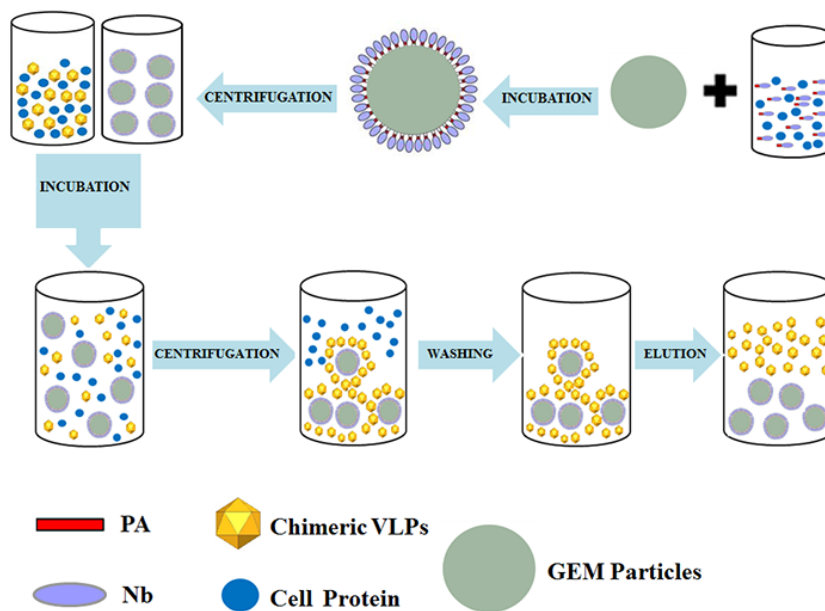
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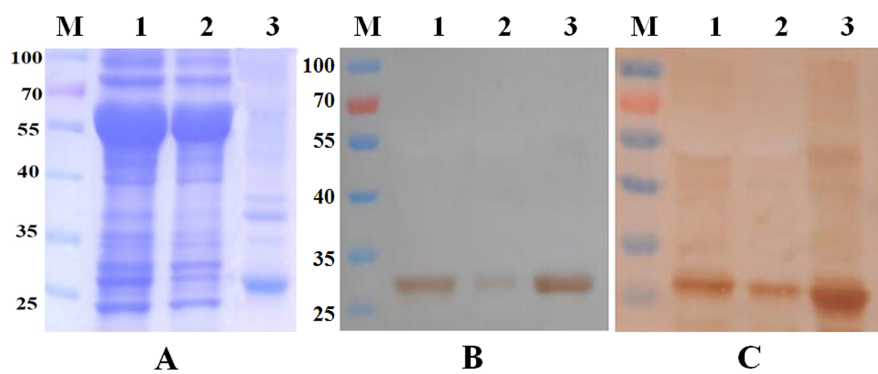
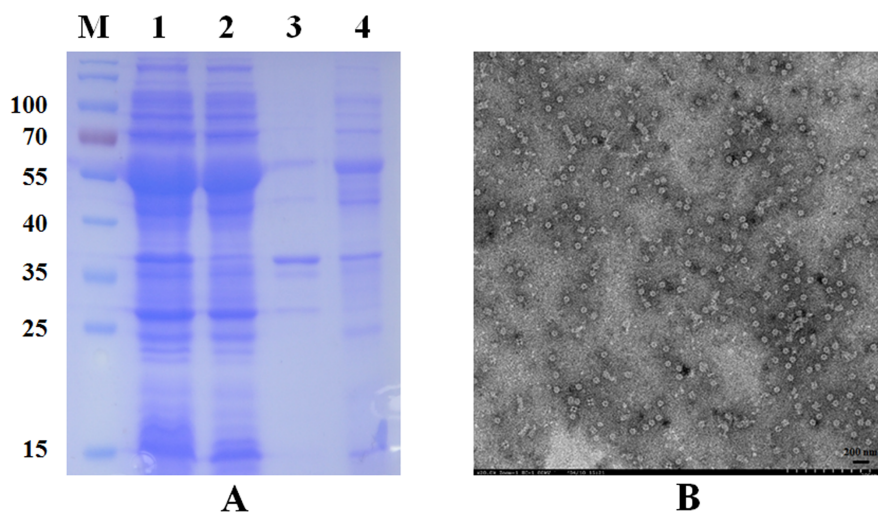
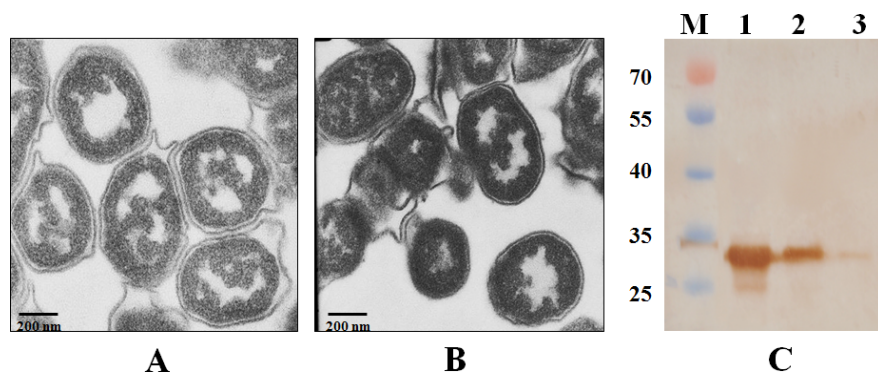


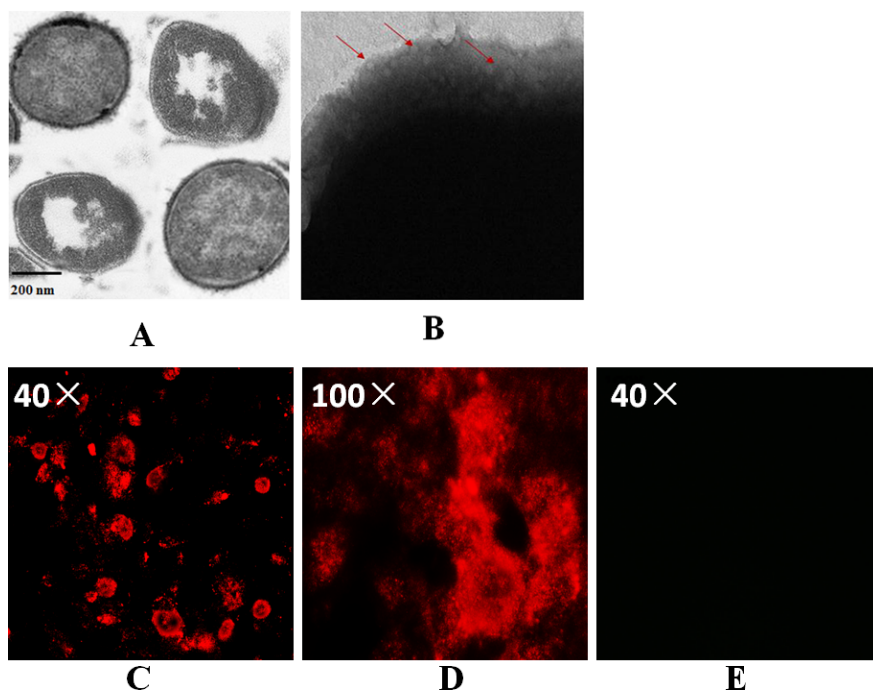
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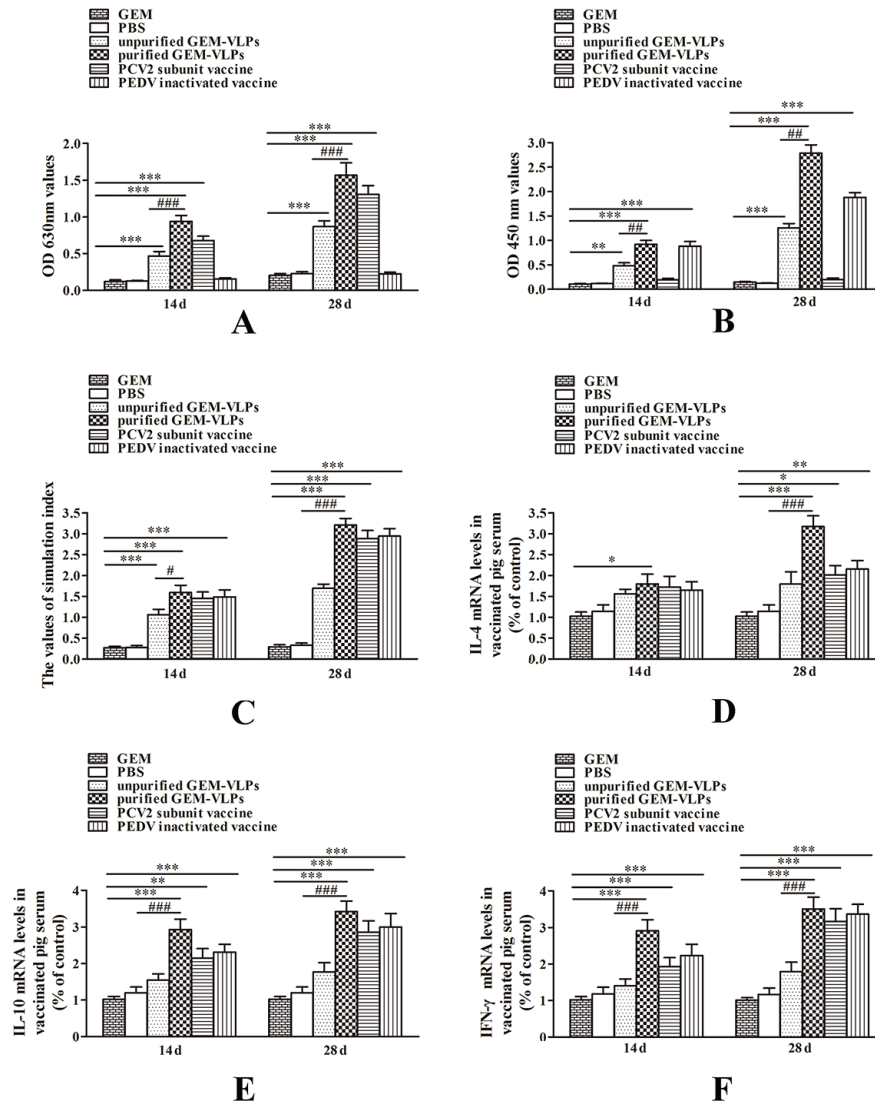


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