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Porcine Circovirus 3 (PCV3) Complete Genome Sequence PCR

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ABSTRACT

This protocol describes four separate PCR runs to amplify the complete genome sequence of Porcine circovirus 3 (PCV3) as four overlapping segments.

EXTERNAL LINK

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MATERIALS

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NAME	CATALOG #	VENDOR
QIAgen DNeasy Blood and Tissue Kit, 50 rxn	69504	Qiagen
MyTaq™ Red Mix 2X master mix		Bioline
RedSafe™ nucleic acid staining solution		iNtRON Biotechnology, South Korea
GelPilot 100 bp Plus Ladder		Qiagen

1 DNA extraction was performed using DNeasy Blood & Tissue Kit extraction kit (Qiagen, Germany) in accordance to manufacturer's instructions. Samples involved include lung, inguinal lymph node, spleen, tonsil, kidney, mesenteric lymph node, heart, liver and brain tissues.

2 Four separate conventional PCR runs were performed to amplify the complete nucleotide sequence of PCV3 as four overlapping segments.

3 Primers, their respective cycling conditions and expected PCR product band size are tabulated below:

Primer Pair	Nucleotide Sequence (5'– 3')	Product Length (bp)	PCR Cycling Condition (Temperature / Time)						Reference
			Initial Denaturation	Number of Cycle	Denaturation	Annealing	Extension	Final Extension	
P1F	CACCGTGTGAGTGGATATAC	854	94ºC / 4 min	35	94ºC / 20 s	55ºC / 30 s	72ºC / 30 s	72ºC / 5 min	Palinski R, Piñeyro P, Shang P, Yuan F, Guo R, Fang Y, et al. A novel porcine circovirus distantly related to known circoviruses is associated with porcine dermatitis and nephropathy syndrome and reproductive failure. J Virol. 2017 Jan 1;91(1):e01879-16.
P1R	CAAACCCACCCTTAACAG								
P2F	GTCGTCTTGGAGCCAAGTG	807	95ºC / 5 min	35	94ºC / 30 s	62ºC / 30 s	72ºC / 1 min	72ºC / 7 min	
P2R	CGACCAAATCCGGGTAAGC								
KF	TTACTTAGAGAACGGACTTGTAACG	649	94ºC / 5 min	35	94ºC / 30 s	55ºC / 30 s	72ºC / 1 min	72ºC / 10 min	Ku X, Chen F, Li P, Wang Y, Yu X, Fan S, et al. Identification and genetic characterization of porcine circovirus type 3 in China. Transbound Emerg Dis. 2017 Jun;64(3):703-8.
KR	AAATGAGACACAGAGCTATATTCAG								
V4F	GAAAACGCGGGAAGCTTGTG	806	95ºC / 5 min	35	94ºC / 30 s	56ºC / 30 s	72ºC / 1 min	72ºC / 10 min	Designed in this study based on PCV3 strain KY075986
V4R	CCACTTCTGGCGGGAACTAC								

4 Type, concentration and volume of reagents used in the four separate PCR runs are the same: 12.5 µL of MyTaq™ Red Mix 2X master mix (Bioline, United Kingdom) and 1.0µM of each primer in a pair, in a 25 µL total PCR reaction volume.

5 PCR products were stained using RedSafe™ nucleic acid staining solution (iNtRON Biotechnology, South Korea) and analysed by agarose electrophoresis using 2.5% agarose gel with GelPilot 100 bp Plus Ladder as marker (Qiagen, Germany).

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