

## 8 Anhang

**Primersequenzen:** Die Orientierung der Oligonukleotide ist in 5'-3'-Richtung aufgeführt.

### PCR-Primer PCV1

Sequenz	Nr.	Temp.	Amplifikat	Position
ATA CGG TAG TAT TGG AAA GGT AGG G	165	61°C	665 nt	441-465
ACA CTC GAT AAG TAT GTG GCC TTC T	166	61°C	665 nt	1130-1106

### PCR-Primer PCV2

Sequenz	Nr.	Temp.	Amplifikat	Position
GGT TTG TAG CCT CAG CCA AAG C	66	62°C	415 nt	172
GCA CCT TCG GAT ATA CTG TCA AGG	67	62,7°C	415 nt	587

### Vektorsequenzprimer

Sequenz	Nr.	Orientierung	Target	Temp.
CTG GCC GTC GTT TTA C	M13	forward	pCR2.1-Vektor	55°C
CAG GAA ACA GCT ATG AC	M13	reverse	pCR2.1-Vektor	55°C

### DD PCR-Primer

Sequenz	Nr.	Primer	Methode
AAG CTT GAT TGC C	AP1	Random	DD
AAG CTT CGA CTG T	AP2	Random	DD
AAG CTT TGG TCA G	AP3	Random	DD
AAG CTT CTC AAC G	AP4	Random	DD
AAG CTT AGT AGG C	AP5	Random	DD
AAG CTT GCA CCA T	AP6	Random	DD
AAG CTT AAC GAG G	AP7	Random	DD
AAG CTT TTA CCG C	AP8	Random	DD
AAG CTT TTT TTT TTT A	H-T <sub>11</sub> A	Anchored	DD
AAG CTT TTT TTT TTT G	H-T <sub>11</sub> G	Anchored	DD
AAG CTT TTT TTT TTT C	H-T <sub>11</sub> C	Anchored	DD

### TaqMan® Primer und Sonden

Sequenz	Nr.	Typ	Target	Temp
Fam-TTC GGC GCC ATC TGT AAC GGT T-Tamra	PCV1	TaqMan Sonde	PCV1, Cap Gen	55°C
Fam-ACG CTT GAC AGT ATA TCC GAA GGT GCG G-Tamra	PCV2	TaqMan Sonde	PCV2, Cap Gen	55°C
AAA AAT GGC ATC TTC AAC ACC	T004B	TaqMan B Primer	PCV2, Cap Gen	55°C
AAT CTC AKC ATG TCC ACC GC	T004F	TaqMan F Primer	PCV2, Cap Gen	55°C
GGC AAT TGA AGA TAC CCG TC	T285F	TaqMan F Primer	PCV1, Cap Gen	55°C
GGC ACA CCC CGC CTT	T286B	TaqMan B Primer	PCV1, Cap Gen	55°C

SYBR Green real-time PCR-Primer

Sequenz	Nr.	Amplifikat	Temp.	Target
TGA ACA TTA TAG AT TTAT CTC CAC A	460 s	173 nt	56°C	IL 18
TGA AGG AAA TGA CAT CAT ATT CT	461 as			
TCA CCC ACA CGG TGC CCA TCT ACG A	468 s	300 nt	68°C	$\beta$ -Aktin
CAC CGG AAG CGC TCG TTG CCG ATG G	468 as			
CAC AGC ATG AGA TGG AAA GG	568 s	109 nt	60°C	10C/SLA
AGA GGC AGC ACT GAT GAC TT	569 as			
AAA TGG AAA TTC CCA GGC TA	572 s	118 nt	56°C	13C/MHC1
CAT TGC CAT GAG CTA TGG TG	573 as			
GCT TTC CAA GTT CTC CAA TG	574 s	102 nt	56°C	14G/DAP5
ATA AGC GTG AAA ATC CCC TC	575 as			
GGC GTA ATC ATG GTC ATA GC	576 s	116 nt	60°C	16A/NSAP1
AGC TCA CTC ATT AGG CAC CC	577 as			
ACT AAT GAC AAA TGC ACT GC	578 s	111 nt	56°C	18A/EHD3
TGA TGG GAA ACC AAA ACA AG	579 as			
CCT ACA CGA CAA CAA CCA AAA C	580 s	150 nt	56°C	26G/Lyncein
GAG AAG GCG GAT GCT GGA	581 as			
CAG CAT TTT CCT CCT CCA TC	633 s	150 nt	56°C	40J
AAC AGA AAC TGG TCC AGC GT	634 as			
CAG AAG GCA CTG AAT GGT	668 s	114 nt	50°C	36 E/Stat3IP
TGA GAA ATG GCC TGT AGT T	669 as			
GTG GGA TTG AGA CGG ACA G	769 s	211 nt	55°C	porcine
TTA ACC CGA GTA AGA ATG TGC	770 as			Caspase3

Northern Blot Sonden

DD-Klon	BLAST	Primer	Fragment
06B	Sus scrofa IL18	T7-Promotor, M13	364 nt
10C	SLA I	M13	332 nt
11C	IL16 premRNA	M13	269 nt
13C	MHC I	M13	305 nt
14G	DAP5	M13	445 nt
16A	NSAP1	M13	273 nt
18A	EHD3	M13	195 nt
20B	EHD3	M13	495 nt
21A	EHD3	M13	194 nt
25J	Lyncein	M13	302 nt
31H	Myosin	M13	225 nt
32J	HexokinaseII	M13	185 nt
33E	Cytokeratin 10	M13	263 nt
34B	ICBP90 transcription factor	M13	243 nt
36E	Stat3 interacting protein/ apoptosis inhibitor like protein	M13	402 nt
37G	MHC I related antigen	M13	261 nt
40J	Unbekanntes Gen	M13	280 nt
41G	Sus scrofa clone	M13	182 nt
$\beta$ -Aktin	$\beta$ -Aktin	468 s/as	300 nt
	Porcine Caspase3	769/770	211 nt

RACE-Primer und –Adaptoren

Sequenz	Nr.	Orientierung	Target	Methode
GCT TTC CAA GTT CTC CAA TG	574	Sense	14G	3'-RACE
ATA AGC GTG AAA ATC CCC TC	575	Antisense	14G	5'-RACE
CTT TGG CTC TTC TAG GGC TT	658	Antisense	14G	5'-RACE
CAT TGG AGA ACT TGG AAA GC	659	Sense	14G	3'-RACE
GGC GTC ATC GTT CAA AT	682	Sense	14G	3'-RACE
ACT GGC AGC AAA GAA GG	683	Sense	14G	3'-RACE
GTG CAC CTG AAG AGC ATA GAG	692	Antisense	14G	RACE
CTT GAC AAT GGA CCA AAG ACG A	699	Sense	14G	RACE
AGG CTT TCC TGA ATG TAT TGG	700	Sense	14G	RACE
GGA GGC GTC TGA ATG TTG TG	773	Sense	14G	5'-RACE
GAT GTT CTC GCA ACT CTA CG	774	Antisense	14G	RACE
GCA GCG ACT CCT CTG AGC T	871	Sense	14G	5'-RACE
TGA GTT CTC GGT GAA GGT ATT	872	Sense	14G	5'-RACE
GGG TGC TTC TCG TTT CAG T	873	Sense	14G	5'-RACE
AAG GGA TGG GAG AAA CGA AA	874	Antisense	14G	5'-RACE
GCC CGA AGA AGC ACT GAA ACG	875	Antisense	14G	5'-RACE
CAG CAT TTT CCT CCT CCA TC	633	Sense	40J	3'-5'
AAC AGA AAC TGG TCC AGC GT	634	Antisense	40J	3'-5'

Adaptoren- und Primersequenzen:

- **5'-RACE-Adapter (0,3 µg/µl) :**

5'-GCU GAU GGC GAU GAA UGA ACA CUG CGU UUG CUG CGU UUG AUG AAA

- **3'-RACE Adapter :**

5'-GCG AGC ACA GAA TTA ATA CGA CTC ACT ATA GGT 12 VN

- **5'-RACE äußerer Primer 10 µM :**

5'- GCT GAT GGC GAT GAA TGA ACA CTG

- **5'-RACE innerer Primer 10 µM:**

5'- CGC GGA TCC GAA CAC TGC GTT TGC TGG CTT TGA TG

- **3'-RACE äußerer Primer 10 µM :**

5'- GCG AGC ACA GAA TTA ATA CGA CT

- **3'-RACE innerer Primer 10 µM :**

5'- CGC GGA TCC GAA TTA ATA CGA CTC ACT ATA GG

Primer zum Nachweis eukaryoter DNA (Kontroll-PCR)

Sequenz	Nr.	Amplifikat	Temp.	Target
TCA CCC ACA CGG TGC CCA TCT ACG A	468 s	300 nt	68°C	β-Aktin
CAC CGG AAG CGC TCG TTG CCG ATG G	468 as	300 nt	68°C	Porcin

Fusionsplasmide und Plasmide rekombinanter Virusgenome

<b>Plasmid</b>	<b>Funktion</b>	<b>Protein</b>	<b>Referenz</b>
pIC1	infektiöser Klon PCV1		Genom von PCV1 mit Überlappung von 77 nt über Pos. 1182-1:1759-1105 im Vektor pUC18
pIC2	infektiöser Klon PCV2		Genom von PCV2 mit Überlappung im Vektor pUC18
pSK144	kloniertes Genom PCV1		Genom von PCV1 im Vektor pSK
pPCV2	infektiöser Klon PCV2		Dr. D. Mahe, Ploufragan, France
pDsRed1N1	Leervektor	DsRed (rot)	Novagen, USA
pDsRedREP'-PCV1	Expression des gespleißten Rep-Transkriptes von PCV1 unter SV40-Promotor	DsRedREP' (rot)	(Mankertz und Hillenbrand, 2001)
pDsRedREP-PCV1	Expression des Volllängen Rep-Transkriptes von PCV1 unter SV40-Promotor	DsRedREP (rot)	(Mankertz und Hillenbrand, 2001)
pDsRedCAP-PCV1	Expression des Capsid-Transkriptes von PCV1 unter SV40-Promotor	DsRedCAP (rot)	(Mankertz und Hillenbrand, 2001)