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(54) CIRCOVIRUS SEQUENCES ASSOCLATED WITH PIGLET WEIGHT LOSS DISEASE (PWD)
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## ABSTRACT

The genome sequences and the nucleotide sequences coding for the PWD circovirus polypeptides, such as the circovirus structural and non-structural polypeptides, vectors including the sequences, and cells and animals transformed by the vectors are provided. Methods for detecting the nucleic acids or polypeptides, and kits for diagnosing infection by a PWD circovirus, also are provided. Method for selecting compounds capable of modulating the viral infection are further provided. Pharmaceutical, including vaccine, compositions for preventing and/or treating viral infections caused by PWD circovirus and the use of vectors for preventing and/or treating diseases also are provided.

FIG. 1
Leu Ala Ser Arg Cys Arg Cys Cys Arg Pro Jeu Thr Leu Ser Phe Ala Leu Cys Tro Arg Val Glu Ala Ala ala Ala Gly Arg Cys Arg *** His Phe Bis Trp Ala Gly Ala Cys Lys Pro Leu Pro Leu Val' Glu Ala Ala Aso Thr Phe Ile Gly Jeu TGG TCG CGT GAA GCC GTC GCC GTC GTG GAG CCG TCG CAG TCA CTT TTA CES TTC 5' ACC AGC GCA CTT CGG CÄG CGG CAG CAC CTC GGC AGC GTC AGT GAA AAT GCC AĂG Thr Ser Ala Lev Arg Gln Arg Gin His Leu Gly Ser Val Ser Glu Asn Ala -ys
Pro Ala His Phe Gy Ser GIy Ser Thr Ser Ala Ala Ser Va Lys Met Pro Ser
Gln Arg Thr Ser Ãa Ala Ala Ala Pro Arg Gln Arg Gin *x* Lys Cys Gln Ala

Ser Phe Arg Gly Aa Va Gly Tyr Ser Thr Pro Thr *** G**** Tyr Asp Iys Leu phe Ala Alà Arg Leu Gly Met Leu pro pro His Glu Gly Lys Ilé Ile Arg Leu Phe Leu Pro Gly Cys Gly Trp Leu Leu His Thr Asn Val Arg Leu Leu Gly GTT CTT $\frac{6 T}{63}$ GCC GGE CGT 12 -GG GGT ATP CTC CAC COA CAA GTG GGA ATT ATM AGG CAA GAA ARG CGG CCC GCA ACC CCA TAA GAG GTG GGT GTT CAC CCT TAA TAA TCC Gin Glu Lys Arg Pro Ala ohr Pro *** Glu Val Gly Val His Pro *** *** Ser Lys Lys Ser Giy Pro Gin Pro His Lys Arg Tro Vêl Phe Tar Leu Asn Asn Pro Arg -ys Ala Ala Arg Asn Pro Ile Arg Gly Gly Cys Ser Pro Leu Ile Ile Ieu

Arg Pro Pro Ser Phe Cys Phe Val Pro Ala Glu Jeu Arg G-y Lys Gln Asn Asn Gly Leu Leu Leu phe val phe Tyr pro jeu Lys mro Aso giy Lys Lys fie ile Glu Ser Ser Ser Phe Phe Leu Ile Arg Ser Ser Giy Tle Glu Arg Lys Ser *** AAG GCT COT CCT CTI TTPT GTT TTA TGC CCT CGA AGG TIA GAG GGA AAA ACT AAT TTC CGA GGA GGA GAA AAA CAA AAI ACE GGA GCT TCC AAT CTC CCT TTT TGA TTA Phe Arg Gly Gly glu Lys Gln Asn Thr Gly Ala Ser Asn Leu Pro Phe *** Leu Ser glu glu glu Lys Asn Lys Tle Arg Glu Leu Pro Ile Ser Leu Phe Asp Tyr fro Arg Arg Arg lys Thr tys Tyr Gy ser Phe Gln Ser Pro Phe Leu Ile fle
Gln Jys His Arg Pro Leu Asn Pro Leu Pro Tyr Phe Glu G.u Gly Gly Pro Thr
Lys Asn Thr Ala Leu phe Thr Gin Phe -eu Thr Ser Ser Arg Val Glu Leu pro
Lys Thr Gin Pro Ser Ser Pro Lys Ser Ser Pro Leu Val Gly *** Arg Trp Pro
AAA ACA AAC ACC GCT CCT ICC AAA CCT TCT CCC ATC TTG AGG AGT GGA GET CCC
TTT TGT ITG TGG CGA GGA AgG TTP GGA AGA GGG TAG AAC TCC ICA COT CCA GGG
Phe Cys Leu Trp Arg Gly Arg Phe Gly Arg Gly *** Asn Ser Ser Pro Pro Gly
Phe tal Cys Gly Gu Glu Gly Leu Glu Glu Gly Arg Thr pro His Leu Gin Gly
Leu Phe Val Âla Arc Lys Val Trp Lys Arg Val glu Leu Leu Thr Ser Arg Gly
Gln Ser Asn Gln *** Ser Ala Ser Lys*** Cys Pro Ser Thr Thr Asn Gln His
Lys Arg Ile Lys Ser Leu Leu Leu Ser ys val Leu His feu pro tle Lys Thr
Asn Ala fhe Lys Ala Leu Phe Cys Val Lys Leu Leu Thr Phe His Tyr Lys Pro
CAA ACG CTT AAA ACE ATP CTT CET CTG AAA ATT GTT CCA CTT CAC CAT AAA ACC 253
gTT TGC GAA TTT TGC TAA GAA GCA GAC TTT TAA CAA GGT GAA GTG CIA TTT TGG
Val cys $---\quad$ Glu Phe cys *** Glu Ala Aso Phe *** Gln Gly Glu Val Val Phe Tro
$\begin{aligned} & \text { Dhe Ala Asn Phe Ala Lys Lys Gin Thr Phe Asn Lys Vat Jys Trp Tyr Phe Gly } \\ & \text { Ieu arg Ile Leu Leu Arg Ser Arg Leu Leu Thr Arg x* Ser Gly Ile Leu Val }\end{aligned}$

FIG. 2a



Ser Gln Leu Arg Ala Thr Giu G-n Leu Thr His Ser Phe Asn Gly Arg Ala Pro His Ser Tyr Gly Leu Leu Lys Arg Tyr Arg Ile His Ser Ile Gle Ala Pro gin Thr Val Thr Ala Ser Cys Asn gly Thr Val Tyr Thr Leu Phe Lys Arg Pro Ser CCA CTG ACA TCG GCT CGT CAA AGG ACA TTG CAT ACA CTC TTT AAA GGC GCC CGA 450 GGI GAC TGI AGC CGA GCA GTT TCC TGI AAC GTA TGT GAG AAA TTT CCG CGG GCT Gly Asp Cys Ser Arg Ala Val Ser Cys Asn Val Cys Glu Lys Phe Pro Arg Ala Val Thr Val Ala Gu gln Phe Pro Va Thr Tyr Val Arg Asn Phe Arg Giy Leu

Gln Val Lys Ser Leu Ser Arg Ser Ser Ala Ala Ala His Asn Ser Ser Leu Gin Ser Phe Lys Gln phe His Ala Pro Jeu His Leu Leu Thr Ile Pro Leu Cys Ser Ala Ser Ser Lys phe Thr Leu pro phe Ile Cys Cys Arg Ser gin fhe Val Ala CCG ACT TGA AAA CTT TCA CTC GCC CTT ----

 Aia Glu Leu Leu Lys Val Ser Gly Lys Met Gln Gln Arg Aso Tro Lys Thr Ala Leu Asn Phe *** Lys *** Ala G-y Arg Cys Ser Ser Val Ile Gly Arg Gln Leu


FIG. 2c
Gly Pro Ile Thr Ser Arg Leu Gln Gln Gly Leu Gln Jeu Leu Glu Arg_Asp Ser G-Y Leu Phe Pro Val Gly *** Ser Ser Asp Trp Ser Tyr Phe Ser Glu Tle Pro Gly Trp Ser his Tyr Glu Glu Val Ala Thr Gly Ala Thr Ser Ala Arg *** Arg GGG GGT CCT TAC CAI GAG GAG TTG ACG ACA GGG TCG ACA TCT TCG AGA GAT AGC 8285 CCC CCA GGA ATG GTA CTC CTC AAC TGC IGT CCC AGC TGT AGA AGC TCT CIA TCG Pro Pro Gly Met Val Leu Leu Asn Cys Cys Pro Ser Cys Arg Ser Ser Leu Ser Pro Gln Glu Trp Tyr Ser Ser Thr Ala Val 3 ro Ala Val Glu Ala Leu Tyr Arg Pro Arg Asn Gly Thr Pro G_n Leu Leu Ser Gln Leu *** Lys Leu Ser Ile Gly
Ser *** *** Lys Ala Ile Lys Ser Ser Gln Gln Ieu Val Ile Tro Pro Pro Val Pro Asn Ser Ser Gin Ieu dys pro Leu Ser Ser Ser Phe Leu Gly Arg Leu Tyr Leu Ile Val Val Lys Cys Asn Gin phe Val Ala ?ro Ser Cys Asp Val Ser Thr CTC CTA ATG ATG AAA CGT TAA AAC CTT CTG ACG ACC TCT TET TAG GTG CCT CCA GAG GAT TAC TAC TTC GCA ATT TIG GAA GAC IGC TGG AGA ACA ATC CAC GGA GGI Glu Asp Tyr Tyr Phe Ala Ile Leu Glu Asp Cys Trp Arg Thr Ile His Gly Gly Arg Ile thr Thr Leu Gln Phe Tro Lys Thr Ala Gly Glu Gin Ser Thr Giu Val Gly Leu Leu Leu Cys Asn Phe Gly Arg Leu Leu Giu Asn Asn Pro Arg Arg Tyr
Arg Ieu Gly Ile Gln Jeu Ieu Pro Gly Val Arg His Gly Lys Gly Net Tyr Phe G-y Phe Ala Ser Lys Phe Cys His Val Trp Gly Thr Gly Lys Glu Trp Jie phe Gly Ser Pro Arg Asñ Ser Ala Tar Ser gly Gly gin Ala Arg Lys gly Tyr Leu TGG GCT TCC GGC TAA ACT TCG TCA CCT GGG TGG GAC ACG GGA AAA GGG TAT ATT ACC CGA AGG CCG ATC TGA AGC AGT GGA CCC ACC CTG TGC CCT TTT CCC ATA TAA -ir Arg Arg Pro Ile *** Ser Ser Gly Pro Thr Leu Cys Pro Phe Pro Ile *** Pro Glu Gly Arg Phe glu Ala Val Âsp Pro כro Cys Ala Ieu Phe Pro Tyr Iys Pro Lys Ala Asp Leu Iys Gin Trp Thr His Pro Val Pro Phe Ser His Ile tys
Leu Asn Ser Leu Arg Iys Gin *** *** Met Thr Ile Thr Lys Ile Iys Ile *** Tyr Ile Val Ser Asp Lys Lys Asn Asp Cys Arg Leu Pro Lys *** Lys *** Glu Ile Phe *** Gln Thr Lys Lys Thr Ile Val Asp Tyr His Asn Lys Asn Lys Asn
TTA ITT AAT GAC TCA GAA AAA ACA ATA GIG TAG CAT TAC CAA AAA TAA AAA TAA AA- AAA TTA CTG AGT CTT TTT TGT TAT CAC ATC GTA ATG GTT TTT ATT TTT ATT
Asn Lys Leu Ieu Ser Iev Phe Cys Tyr His Ile Val Met Val Phe Ile Phe Ile İe Asn Tyr *** Val Phe Phe Val tle Thr Ser *** Tro Phe Leu Phe Leu Phe Ile Thr Glu Ser Phe Leu Leu Ser His Arg Asn Gly Phe Tyr Phe Tyr Ser
Lys Ser Pro Arg Glu Pro Tyr Ile Arg Gln Ile Thr Cys Leu Tyr Asp Val Lys Asn Leu Pro Asp Lys Leu Ile Phe Glu Arg The Gin Val Tyr Ile Thr Leu Arg Met *** Leu Thr Lys $* * *$ Ser Leu Asn Glu Ser Asn Tyr Met Phe Leu *** Gly GTA AAT CTC CCA GAA AGT CCT ATT TAA GAG ACT TAA CAT GTA TTT ATC AGT TGG
 CA. TTA GAG GGT CTI TCA GGA TAA ATI CTC TGA ATT GTA CAT AAA TAG ICA ACC
His Leu Glu Gly Leu Ser Gly *** Ile Leu *** Ile Val His Lys *** Ser Thr
İe *** Arg Val Phe Gln Aso lys Phe Ser Glu Leu Tyr Ile Asn Ser Gln ?ro Phe Arg Gly Ser Phe Arg Ile Asn Ser Leu Asn Cys Thr *** Ile Val Asn Leu.



Cys Ser Thr Leu Val Trp Val Phe Lys Trp Ser His Ser Trp Phe Leu Leu Leu
Lys Ala Pro Val Jeu *** Asn Asn Pro Arg Ala Arg Thr Gln Pro His Leu Val
Asn ?ro Gln Phe Trp Asp Ile Thr Gln Asp Leu Glu Pro Lys Pro Thr Phe Tyr
Ile Gln Ser Ser Gly Ile Jeu G_n Lys Thr *** Ser Gin Asn Pro Pro Ser thr
AIA AAC CGA CCI TGG -----
tat tig gct gaa acc ant can tict tit get cta gct cte git tgg gge tga ag
Iyr Leu Ala Gly Thr Asn Gln Leu Phe Gly Leu Ala Leu Val Tro Gly *** Ser
He Trp Leu glu Pro Ile Asn Cys Leu Val *** Leu Tro Phe gly Gly Gly Val
Fin Gly Tro Asn Gln Ser Ile Val Trp Ser Ser Ser Gly Leu Gly Val Lys Tyr
Gln Leu Pro Leu Tyr Leu Ala Å Lys His His Pro Pro Leu Leu_Leu *** Fyr Arg See fis Tyr Thr Phe Pro Gin Arg Tle Thr His Arg Ser Ser -yr Asn Iie Gly Pro Thr Thr Pro Leu Pro Ser Gly *** Fro Thr Ala Pro Pro Thr Thr Leu IEG ACC TCA CCA TCC ATT TCC CGA CGG AAP ACC ACA CCO CCC TCC TCA TCA AAT
 Thr Tro Ser Gly Arg *** Arg Ala Ala Teu Tro Cus Gly Gly Aro Ser Ser **x


Leu 3 ro *** Leu Gly Leu Gln His Leu Pro Asn Cys Leu Gln Cys Gly Leu Tyr
Tyr Pro Asp Tyr Ala Leu Asn Thr Ser Pro Thr Val Phe Asn Ala Asp Leu Tle
ILe Pro Thr Met Pro Tro Thr Pro Pro Pro Pro *** Leu Thr Pro Met Trp Ser
ATA TCC CCA GTA TCC GGT TCA ACC ACC TCC CCC AAT GTT TCA ACC GTA GGI TCT
IAT AGG GGT CAT AGG CCA AGT TGG TGG AGG EGG TTA CAA AGT TGG CAI CCA AGA
Iyr Arg Gly His Arg Pro Ser Trp Tro Arg Gly Ieu Gln Ser Trp His Pro Arg
Ile Gly Val Ile Gly Gln Val Gly Giy Gly Giy Tyr Lys Val Gly Ile Gln asp
Cys Cys His Val Trp Cys Arg Lys Ser *** Leu His His Pro Arg Gln Pro Leu Val Val hr Ser gly val Gly Arg Gin Asn Ser Thr Ile Pro Asp Arg pro Tyr Ieu Leu Leu Pro Gly Leu Val Glu Lys Ile Leu Pro Ser Pro Thr Glu Pro Thr ATT GTT GGC ACC TGG GTT GIG GAG AAA CTA ATC, TCC ACT ACC CCA GAG ACC CCA 1359 TAA CAA CAG TGG ACC CAA CAC CTC TTT GAT TAG AGG TGA TGG GGT CTC ПGG GGT Gln Gin Trp Thr Gln Fis Leu Phe Asp *** Arg *** Tro Gly Leu Frp Gly Asn Asn Ser Gly Pro Asn Thr Ser Leu Tle Arg Gly Asp Gly Val Ser Gly Val
Thr Thr Val Asp Pro Thr Pro Leu $\star \star \star$ Leu Gul Vat Gly Ser Leu Gly ***
Ile *** Ile *** Gly Lys *** Myr Pro Ieu Ile Pro Phe Thr Pro Thr Pro Pro Phe Glu Tyr Lys Ala lys Arg Tie Arg Tyr Tyr Gin Phe Pro Leu Pro Leu Pro The Asn Met Asn Jeu Arg Glu Leu Val Thr Thr Asn Ser Leu Tyr Pro Gyr Pro TTT IAA GTA TAA ATC GGA AAG ATM ATG CCA TCA, TAA CCT TTC CAT CCC CAT CCC AAA ATT CAT ATT TAG CCT TTC TAA TAC GGI AGT ATT GGA AAG GTA GGG GTA GGG Iys Ile His Ile *** Pro Phe *** Tyr Gly Ser Ile Gly Lys Val Gly Val Gly Lys Phe Ile phe Ser Leu Ser Asn thr Val Va Leu Glu Arg *** Gy *** Gly
Asn Ser Tyr Leu Ala Fhe Leu -le Arg *** Tyr Trp Jys Gly Arg Gly Arg Gly
GIn His Arg Arg Leu Pro Pro Pro Val Pro Arg His Gln Ile Glu A a Arg *** Asn Thr Gly Gly Ser Pro Pro Led Phe Gln Gly Ile Asn Phe Arg Leu Glu Asn Thr Pro Ala Ala Gln Pro Pro Ser Ser Ser Ala Ser Thr Ser Asp *** Ser Thr CCA ACC ACG GCG GAC TCC CCC CCO CCI TGA CCG GCT ACA ACT TAG AGT CGA GCA GET IGG TGC CGC CTG AGG GGG GGA GGA ACT GGC CGA TGT TGA ATC TCA GCT CGT Gly $\operatorname{Irp}$ Cys Arg -eu Arg Gly Gly Gly Thr Gly Arg Cys *** Ile Ser Ala Arg Val Gly Ala Ala *** Gly gly Giu Giu Leu Ala Åp Val glu Ser Gin Leu Val Leu Val Pro Pro Glu gly gly Arg Asn Trp Pro Met deu Asn Leu Ser Ser Leu

> Cys Glu Leu Ile Ala Ala Leu Gir Arg Arg Lys His His Thr Cys Ile Arg ***
Val Asn mrp Ser pro Gln Ser His Gly gly Arg tie Thr Leu Val Phe Glu Arg Leu Met Gly Leu His Ser Arg Thr Asp Glu Glu *** Pro Ser Tyr Leu Asn Glu ATT GTA AGG ITC TAC CGA CGC TCA CAG GAG GAG AAT ACC ACT CAT GTT СAA GAG TAA CAT TCC AAG ATG GCT GCG hg- GTC CTC CTC TTA TGG TGA GTA CAA ATT CTC *** His Ser Lys Net Ala Ala Ser Val Leu Leu Leu Trp *** Val Gln Ele Leu Asn Ile Pro Arg Tro Leu Arg Val Ser Ser Ser Tyr Gly Glu Tyr Lys Phe Ser
Thr Phe Gln Asp Gly Cys glu Cys Pro Pro Leu Met Val Ser Thr Asn Ser Leu
Phe Pro Pro Phe Gln Ieu Tyr Gly Asp Iys Pro Ala Met Gln Jeu Pro Lys Gln Ser Leu Arg Ser Asn Phe Tle G-y Thr Lys Arg Arg Trp Arg Tyr Arg Asn Arg Leu Phe Aa Pro Ile Ser Ser Va, Arg Arg Glu Ala cly Aso Thr Val mar Glu ATC ITT CCG CCC TTA ACT TCT ATG GGC AGA AAG CCG CGG TAG ACA TTG CCA AAG tag ana ggc gge ant tga aga tac ccg tct tic ggi gcc atc tgi nac gat tic *** Lys G-y Gly Asn *** Arg Tyr Pro Ser Phe Gly Ala Ile Cys Asn Gly Phe Arg Lys Ala Gly Ile Glu Asp Thr Arg Leu Ser Ala Pro Ser Val Thr Val Ser
Glu Arg Arg Giu Leu Iys Ile Pro Val Phe Arg Arg His Leu *** Arg Phe Leu


FIG. 2g


FIG. 3a


FIG. 3b

| circopormank circobormeeh circopordfp |  |  | $\left\lvert\, \begin{gathered} 1240 \\ \begin{array}{c} T G G G G G G A A \\ T \in G G G G G A A \\ T G G G G T G A A \end{array} \end{gathered}\right.$ |  | 1250 1250 1250 |
| :---: | :---: | :---: | :---: | :---: | :---: |
| circcpormank circooormeeh circopordfp |  |  | $\begin{array}{r} 1290 \\ \left.\begin{array}{c} \text { CGGGAGAGD } \\ C G G A G E A G F \\ C G G A G A G I \end{array} \right\rvert\, \end{array}$ |  | 1300 1300 1300 |
| circopormank circopormeeh circopordfp |  | $\begin{array}{r} 1330 \\ \frac{T G G A G G G G T}{T G G G G G G T} \\ T G G A G G G G T \end{array}$ |  |  | 1350 1350 1350 |
| circopormank <br> circopormeeh <br> circopordfp |  |  | $\begin{array}{r} 139 \\ \text { GAGIA } \\ \text { GAGGA } \\ \text { GAGIGA } \end{array}$ | $\begin{array}{r} 1400 \\ \text { GGGTCTCG } \\ \text { GGTCTCG } \\ \text { GGOTCTCTG } \end{array}$ | 1500 1500 1500 |
| circcoorman: <br> circopormeeh <br> circcpordfp |  |  |  | $\begin{array}{r} \frac{1450}{\text { GGAAGGTAG }} \\ \text { GGARAGGTAG } \\ \text { GGARGGTAG } \end{array}$ | $1 \angle 50$ 1250 1550 |
| circcoormank <br> circcoormeeh <br> circoocrdfp |  | $\begin{gathered} 1480 \\ T G G G G G G G \\ T G G G G G G G \\ T M A G G G G G \end{gathered}$ | $\begin{gathered} 1490 \\ \operatorname{AGGAACTGO} \\ G G G A R C T G O \\ G G A A C T G O \end{gathered}$ | 1500 <br> CGATGTTGA cgatgitga CGATGITGA | 1500 1500 1500 |
| circooormenk <br> circcoormeeh <br> circopordip |  |  | $\begin{aligned} & 1540 \\ & \text { CGAGTATCC } \\ & \text { CGGTATCC } \\ & \text { CGAGTCCC } \end{aligned}$ | $\begin{array}{r} 1550 \\ C C=T T T A G G \\ C C T T T A G \\ C C C T R T G \end{array}$ | 1550 1550 1550 |
| circoporman: <br> circopormeeh <br> circcoordfp |  |  |  | $\begin{aligned} & 1600 \\ & \begin{array}{c} C G T C T T T C G \\ C G C T T T G \\ C G C T T C G \end{array} \\ & \hline C G T \end{aligned}$ | 1600 1600 1600 |
| circooormenk <br> circooormeeh <br> circopordfp |  | $\begin{aligned} & 1630 \\ & \begin{array}{l} \text { GAAGGCGGG } \\ \\ \text { GAAGGCGGG } \end{array} \\ & \hline G A G G G G G \end{aligned}$ |  |  | 1650 1650 1650 |
| circcoormank <br> circopormeeh <br> circopordfp |  |  | 1690 $C G G G I C D$ $C G G G I C N$ $C G G G C C F$ | $\begin{array}{r} 1700 \\ C T T C T G C G G \\ C T T T G G G \\ C T T C T G G G \end{array}$ | 1700 1700 1700 |
| circooormans <br> circcoormeeh <br> circooordfp |  |  | $\begin{gathered} 1740 \\ \begin{array}{l} \text { AAGTGAAAGA } \\ \text { AGGTGAAGA } \\ \text { RGTGAAGAA } \end{array} \end{gathered}$ | $\begin{array}{r} 1750 \\ \begin{array}{l} \angle G T G C G C T C C \\ A G T O C C T C C \\ A G T C G C T G C \end{array} \end{array}$ | 1750 1750 1750 |
|  | 1770 | 1780 | 1790 | 1800 |  |
| circooormeeh circopordfp | 1751 TGTAGPATM. |  |  |  |  |

FIG. 3c


FIG. 4


FIG. 5


FIG. 6
-eu Ala Ser Arg Cys Arg Cys Cys Arg Pro Ieu Val Glu Ala Ala Val His Gly Trp Arg Val Glu Ala Ala Ala Ala Gly Arg Cys Cys Arg Leu Leu Leu Net Gly Gly Ala Cys Lys Pro Leu Pro Leu Val Glu Ala Ala Gly *** Cys Cys Cys Ala - TGG TCG CGT GAA GCC GTC GCC GTC GTG GAG CCG TCG TGG AGT CGT CGT TET ACG 5' ACC AGC GCA CTT CGG CAG CGG CAG CAC CIC GGC AGC ACC ICA GCA GCA ACA TGC Thr Ser Ala Leu Arg Gin Arg Gln His Leu Gly Ser Thr Ser Ala Ala Thr Cys
Pro Ala His Phe GIy Ser Gly Ser Thr Ser Ala Ala Pro Gin Gln Gln His Ala
Gln Arg Thr Ser Âa Ala Ala Ala Pro Arg Gln His Leu Ser Ser Asn Met Pro
Ala Leu Leu Ile Ser Ser Ala Ser Gly Ieu Gly Met Phe Pro Pro His Glu Ser
Leu Leu Phe Phe Pro Leu Ieu Pro Gly Tro Gly Trp Leu Jeu His Thr Asn Val Trp Cys Ser Ser His Phe Phe Arg Val Gly Val Gly Tyr Pae Thr Pro Thr *** GgT CGT TCT TCT TAC C $\quad \frac{1}{72}$ CTT CGC CTG GGG TTG GGG TAF ITT CCA CCC ACA AGT CCA GCA AGA AGA ATG GAA GAA GCG GAC CCC AAC CCC ATA AAA GGT GGG TET TCA Pro Ala Arg Arg Met Gu Glu Ala Asp Pro Asn Pro Ile Lys Gly Giy Cys Ser
Gln gln Glu Glu Trp Lys Lys Arg Thr Pro Thr Pro *** Lys Val Gly Val. His
Ser Lys Lys Asn Gly Arg Ser Gly Pro Gln Pro His Lys Arg Trp Val Phe Thr
Gln Ile Ile Arg Gly Phe Val Leu Ala Ieu The Tyr Pro Ile Lys Trp Tyr Gly Arg Phe Leu Gly Glu Ser Ser Ser Arg Leu phe Ile Arg Ser Arg Gly Ile Asp Glu Ser Tyr Asp lys Arg Leu Arg Ala Cys Ser Phe Val Pro Aso Glu Leu Ile GAG ACT TAT TAG GAA GGC TTC TGC TCG CGI TCT TTT ATG CCC TAG AAG GTT ATA CIC TGA ATA ATC CTM C̄CG AAG ACG ĀCC GCA AGA AAA TAC GGG ATC TIC CAA TAT --- --- --- --- --- --- --- --- --- --- --- -- -Leu *** Ile Ile Leu. Pro Lys Thr Ser Ala Arg Lys Tyr Gly Ile Phe Gln Tyr Ser Glu *** Ser Phe Arg Arg Arg Ala Gln Glu Asn Thr Giy Ser Ser Asn fle -eu Asn Asn Pro Ser Glu Asp Glu Arg Iys Iys Ile Arg Asp Leu Pro Ile Ser
*** Lys Ile Tle Lys Asn Asn Ala Leu Ieu Thr Ile Leu Phe Ser Ser Cys Arg Arg Asn Ser *** Lys ${ }^{*} \bar{x}$. Thr Pro Ser Ser Pro Leu Ser Ser Pro Arg Val Gly Gly Tle Gln Asn Asn * $\bar{x} *$ Gin Gin Arg Pro Pro Tyr His Pro Leu Val Phe Val
 CCC TAT TTG ATT ATT TTA TTG TTG GCG AgG AgG gTa ATG AGG AAG gac gan Cac Pro Tyr Leu Ile Ile Leu Leu Leu Ala Arg Arg Val Met Arg Lys Asp Glu His

-eu Phe Aso Tyr Phe fle Val Gly GIu Glu gly Asn Glu Giu Gly Arg Thr Pro
Val Glu Leu Pro Glu Ser Ile Lys His Jeu Ieu Leu Ser Lys Ile Phe His Deu *** Arg Trp Pro Asn Ala Leu Lys Thr Phe Phe Cys Val Lys Leu Leu Thr Phe Glu Gly Gly Pro Thr Arg *** Asñ Gin Ser Ser Ala Ser Lys *** Tyr Leu Ser

Pro Ile Gln Thr Gly Ala Ala Val Asp Ieu Phe Arg Phe Ser Cys Ile Leu Leu His Tyr Lys Pro Ala Arg Gln Trp Met Ser Phe Ala Phe Pro Val Ser *** Cys Thr Thr Asñ Pro His Gly Ser Gly Cys Arg Ser Leu Ser Ieu Phe Leu Asp Alà
 AGT GGT ATT TGG GTG CCC GCT GCC ACA TCG AGA AAg CGA AAG GAA CAG ATC AGC Ser Gly Ile Trp Val Pro Ala Ala Thr Ser Arg Iys Arg Iys Gli Gln Ile Ser Val Val Phe Gly Cys Pro Leu Pro His Arg Glu Ser Glu Arg Asn Aro Ser Ala Trp Tyr Leu Gly Ala Arg Cys His Ile Glu Lys Ala Lys Gly Thr Aso Gln Gln
Ile Phe Phe Val Ala Thr Phe Phe Ala Val *** Gln His Leu Thr Ser Ser Arg
Phe Leu Ser Tyr Gln Leu Leu Ser Pro Leu Lys Ser His Ser His Pro Ala Gly
Ser pyr Leu fle Ser Cys Tyr Leu Leu Cys Ser val Ser Pro thr His Leu Glu
AGA ATA AAG AAT ACT GCA GIA AAG AAG GCA ACT TAC TGA TGG AGT GTG GAG CTC
Arg Ile Lys Asn Thr Ala Val Lys Lys Ala Thr Tyr *** Tro Ser Val Glu Leu
$\begin{aligned} & \text { GIu *** Arg Ile Leu Gin *** Arg Arg Gln Leu Thr Asp Gly Val Trp Ser Ser } \\ & \text { Asn Lys Glu Tyr Cys Ser Lys Glu Gly Asn Leu Leu Met Glu Cys Gly Ala Pro }\end{aligned}$ Ser Arg Ieu Ser Ieu Pro Thr Val Gln Arg Ser Ser His Thr Gly Gin Gln Leu Leu Asp *** Pro Cys Arg Leu Ser Arg Asp Val Ala Thr Leu Val Lys Asn Ser *** Ile Glu Pro Val Val Ser his Gly Thr ${ }^{* * *}$ GIn Gin Ser Tyr Arg Thr Pro GAI CTA GAG TCC CIG TTG CCT CAC TGG ACA GAT GAC GAC ACT CAT GGA ACA ACC CTA GAT CTC AGG GAC AAC GGA GTG ACC TGT CTA CTG CIG TGA GTA CCT TGT TGG Leu Asp Leu Arg Asp Asn Gly Val Thr Cys Leu Leu Jeu *** Val Pro Cys Trp *** Ile Ser Gly Thr Thr Glu *x* Pro Val Tyr Cys Cys Glu Tyr Lev Val Gly Arg Ser Gln Gly Gln Arg Ser Asp Leu Ser Thr Ala Val Ser Thr Leu Leu Giu
Ala Pro Thr Gln His Gly Asn Cys Leu Leu Val Arg Tyr Arg Lys Aso Ser Ile Leu Pro Leu Arg, Thr Val Thr Ală Ser Cys Cys Gly Thr Val Asn Thr Teu Phe Ser Arg Ser Asp Pro Ser Arg Gln Leu Ala Ala Gly Gin Ieu Thr Gin *** Phe

 Arg Ala Gly Val Tro *** Pro Leu Gln Ser Ser Thr Jeu *** Aro Leu Ser Glu Glu Arg Glu Ser gly Aso Ary Cys Arg Ala Ala Pro Cys Asn Val Cys Gin Lys Ser Gly Ser -eu Val Thr val Ala Glu Gin His Pro Val Thr Phe Val Arg Asn

Glu Ala Pro Gln Ser Phe Iys Gln Phe His Ala Pro Phe His Leu Leu Thr Ile Lys Arg Pro Ser Ala Ser Ser Lys Phe Thr Leu Pro Phe Ile Cys Phe Arg. Ser Asñ gly Arg Ala Pro gln Val lys Ser Leu Ser Arg Ser Phe Ală Ser Ala His TAA AGG CGC CCG ACC GAC TTG AAA ACT TTC ACT CGC CCT TTT ACG TCI TCG CAC ATT TCC GCG GGC TGG CTG AAC ITT TGA AAG TGA GCG GGA AAA TGC AGA AGC GTG
Ile Ser Ala Gly Tro Leu Asn Phe *** Lys *** Ala Gly Iys Cys Arg Ser Val Phe Pro Arg Ala Gly *** Thr Phe Glu Ser Glu Arg Glu Asn Ala Giu Ala *** Phe Arg Gly Jeu Ala Glu. Leu Leu Lys Và Ser Gly Lys Met Gln Iys Arg Asp

Pro Leu Ser Ile Tyr Val Asp Asn His Pro Tro Arg Pro Thr Thr Phe Ala Phe GIn Phe Val Leu Thr Cys Thr Met Thr Pro Gly Gly Pro His Pro Leu Jeu Leu. Asn Ser Ser ${ }^{* * *}$ His Val Arg *** Gin Pro Ala Val Gin Thr His Iyr Phe Cys TAA CCT TCT GAT TAC ATG TGC AGT AAC ACC CCG GTG GAC CCA CAC CAT TMT CGT ATI GGA ĂGA CTA ATE TAC ACG ICA TTG TGG GGC CAC CIG GGI GIG GTA AAA GCA Ile Gly Arg Leu Met Tyr Thr Ser Leu Trp G-y His Leu Gly Val Val Lys Ala Jeu Glu Asp **x Cys Thr Arg His Cys gly Ala Thr Tro Val Tro *** Jys Gin
Trp Lys Thr Asn Val His Val Ile Val Gly Pro Pro Gly Cys Gly Jys Ser Lys
Pro Ser Ser Ile Lys Cys Val Arg Phe Gly Cys Val Pro Phe Trp Arg Ser Val His Ala Ala Leu Lys Ala Ser Gly Ser Val Val Tyr Gln Phe Gly Gly eu phe Ile Pro Gln *** Asn Gln Leu Gly Pro Phe Trp Met Ser Ser Val Val *** Phe TTA CCC GAC GAT TAA AAC GIC TGG GCC TTT GGT GTA TGA CCI TAG GTG GAT CTT AAT GGG CTG CPA ATT TTG CAG ACC CGG AAA CCA CAT ACT GGA AAC CAC CTA GAA Asn Gly Leu Leu Ile Leu Gln Thr Arg Lys Pro His Thr Gly Asn His Leu Glu Met Gly Cys **x Phe Cys Arg Pro GIy Asn His Ile Leu Guu Thr Thr *** Iys Irp Ala Ala Asn Phe Ala Asp Pro Glu Thr Thr Tyr Trp Lys Pro Pro Arg Asn

Ieu Pro Pro Ile Thr Val Met Thr Phe Phe His Asn Asn Asn Ile Val Iys Ile Jeu His His Ser Pro *** Trp Pro Ser Ser Thr Thr Thr Tle Ser Ser ys *x Cys Thr Thr Pro His Asn Gly His His Leu Leu Pro Gin *** Gin His Ser Lys
 ACA AGT GGT GGG ATE GTT ACC ATG GTG AAG AAG -GG TTG TTA TTG ATG ACT TTN Thr Ser Gly Gly Met Val Thr Met Val Lys Iys ---------- Ieu Leu Leu Met Thr Phe Gln Val Val gly Tro Leu Pro Tro *** Arg Ser. Gly Cys Tyr *** *** -eu Leu lys Trp Trp Asp Gly Tyr His Gly Glu Gilu Va. Val Val tle Asp Asp Phe Tyr
Ala Pro Gln Giy Pro Ile Ile *** Gln Ser Gln Thr Ile Ser Ile Trp Gln Ser Pro Gln Ser Gly Gin Ser Ser Arg Ser Jeu Ser His Ser Arg Dyr Gly Asn Val His Ser Ala Ala Arg Pro His Asp Val Ser Val Mr His Aso Iie Asp Met Ser TAC CGA CCG ACG GGA CCC TAC TAG ATG ACT CTG ACA CAC TAG CTA TAG GTA ACT ATG GCT GGC IGC CCT GGG ATG ATC TAC TGA GAC -GT GIG ATC GAT ATC CAT TGA Met Ala Gly Cys Pro Gly Met Ile Tyr *** Asp Cys Val Ile Asp Ile His *** Tro Leu Aia Ala Leu G1y *** Ser Thr Glu Gor Val *** Ser Tle Ser Ile Asp
Gly Tro Leu Pro Trp Asp Asp Jeu Leu Aro Leu Cys Asp Arg Tyr Pro Leu Thr Tyr Leu Ser Phe Thr Ser Ser Tyr Arg Lys Gln Gly Ala Thr Asn Gln Asn Gly
Thr Ser Val Leu Pro pro Val Thr Gly Lys Lys Ala Arg Leu Tle Arg Ile Val Gln Leu Ser $\times x *$ Leu His Phe gin val Lys Lys Pro gly Cys Tyr Glu Ser ***
 CTG TAG AGA CTA hag gTg gai ctg tac CTT TTT -GG CCC GCA GIA TTC TGA TTA Leu *** Arg Leu Lys Val Glu Leu Tyr Leu Phe Frp Pro Ala Val Phe *** Leu
Cys Arg Asp **x Arg Tro Asn Cys Thr Phe Pie Gly Pro Gin Tyr Ser Asp Tyr
Val Glu Thr dys Gly Gly Thr Val Pro phe Leu Aadarg ser fle -eu Ile thr
FIG. 8c
Ala Ile Ieu Gly Arg Gln Phe Pro Val Gly *** Ser Ser Asp Trp Ser Tyr Phe Leu Leu *** Val Gly Asn Ser His Tyr Glu Glu Val Ala Thr Gly Ala Thr Ser Trp Cys Asp Ser Gly Thr Pro Ile Thr Ser Arg Leu Gln Gln Gly Ieu Gln Leu GGT CGI TAG TCT GGG GCA ACC TTA CCA TGA GGA GTT GAC GAC AGG GTC GAC ATC CCA GCA ATC AGA CCC CGI TGG AAT GGI ACT CCT CAA CTG CTG TCC CAG CTG TAG Pro Ala Ile Arg Pro Arg Tro Asn Gly Thr Pro Gln Leu Jeu Ser Gln Leu *x* Gin Gin Ser Asp pro Val gly Met Val Leu Leu Asn Cys Cys Pro Ser Cys Arg Ser Asn Gin Thr Fro Leu Giu Trp Iyr Ser Ser Thr Ala Val Pro Ala Val Giu

Gly Arg Leu Phe Fro Ala Leu Glu Asp Gly Lys Gly Gly Tro Ala Arg Phe Lys Asp Val Ser Ser Pro Pro Trp Asn Thr Val Arg Glu Gly Gly His Gly Ser Asn Ile Trp ?ro Pro Leu Pro Gly Thr Arg *** Gly Lys Gly Gly Met Gly Gln I_e
 AAT CCA CGG AGG AAg GGg GCC AGI TCO TCA CCC TTT CCC CCC CAT GCC CIG AAT Asn Pro Arg Arg Lys Gly Ala Ser Ser Ser Pro Pae Pro Pro His Ala Leu Asn Tle His gly gly Arg Gly Pro Val Aro His Pro Phe Pro Pro Met Pro ***
Ser Thr Gle
Glu Gly Gly Gln Phe Val Thr Leu Ser Pro Pro Cys Pro Glu Phe
Trp Ile Phe Tyr Ile Val Ser Asp Lys Lys Asp Ser Arg Ieu Pro Lys *** *** Gly Tyr Ser Ilé Phe *** Gln Thr Lys Lys Ile Val Glu Tyr His Asn Lys Asn Glu Met his Phe Leu Asn Ser Leu Arg Lys *** *** Lys Thr Ile Thr Lys İe AAG GIA TAC TTT ATT TAA TGA CIC AGA AAA AAT AGT GAA GCA TTA CCA AAA ATA TTC CAT ATG AAA TAA ATM ACT GAG TCT TTT TTA TCA CTT CGT AAT GGT TTT TAT Phe His Net Lys *** Ile Thr Glu Ser Phe Leu Ser Leu Arg Asn Gly Phe Tyr Ser Ile *** Asn Lys Ieu Leu Ser Leu Phe Tyr His Phe Val Met Val Phe -le
Pro Tyr Glu Ile Asn Tyr *** Val Pae Phe Ile Thr Ser *** Tro Phe Ieu Leu

[^0]Cys Pro *** Val Ser Ile Thr Asn Arg Thr Thr Tyr Val Thr Lys Ser Arg Leu Val His Asn Cys Pro Tyr Gln Ile Gly Pro Arg Ile Tyr Gin Lys Arg Val Cys Tyr Met Thr Val Arg Ile Asn Tyr Glu Gin Asp Tyr Ile Ser Asn Glu Phe Ala TAT GTA CCA ATG TGC CTA TAA CAT AAG GAC CAG CAT ATA TGA CAA AAG CחT GCG ATA CAT GGT TAC ACG GAT ATT GTA TTC CTG GTC GTA TAT ACT GTT TTC GAA CGC Ile His Gly Tyr Thr Asp Ile Val Phe Leu Val Val Tyr Thr Val Phe Giu Arg Tyr Met Val Thr Arg Ile Leu Tyr Ser Trp Ser Tyr fle Leu Phe Ser Asn Ala Thr Trp Ieu His gly Tyr Cys 1 le Pro gly Arg Ile Tyr Cys Phe Arg Thr gln
Ala Ser Ala *** Thr Thr *** Met Glu Leu Leu Lys Myr Asp *** Gly Cys Ser His Arg Pro Arg Arg Pro Arg Cys Lys Trp Cys Asn Thr Thr Glu Ala Val Ala Thr Gly Leu Gly Val His Asp Val Asn Gly Ala Thr Gln Leu Arg Leu Trp Leu TCA CGE CTC CGG ATG CAC CAG ATG TAA AGG TCG TCA AAC ATC AGA GIC GGI GTC AGI GCC GÄG GCC TAC GIG GTC TAC ATM TCC AGC AGT TTG TAG TCT CAG CCA CAG Ser Ala glu Ala Tyr Val Val Tyr Ile Ser Ser Ser Leu *** Ser Gln Pro Gln Val Pro Arg Pro Thr Tro Ser Thr Phe Pro Ala Val Cys Ser leu Ser His Ser
Cys Arg Gly jeu Arg Gly Leu His Phe Gln Gln Phe Val Val Ser Ala Thr Ala
Thr Glu Lys Thr Thr Gln Asn Ser Thr Ile Leu Leu Ser Ile *** Ser Leu A.sn pro Lys Lys Gin Gin Lys Thr Pro Leu Leu a** Tyr His Phe Arg Pro Cys Thr Gln Asñ Arg Lys Asn Asñ Pro Gln Phe Tyr Asp Ile Thr Phe Asp Leu Val Pro GAC --CTG GTT TCT TTT GTT GTT TGG TTG GAA GTA ATC AAT AGT GAR ATC TAG GAC AG 1242 --- -Leu Val Ser Phe Val Val Tro Leu Glu Val Ile Asn Ser Glu Ile *** Asp Arg Trp Phe Leu Leu Leu Fhe Gly Trp Lys *** Ser Ile Val Lys Ser Arg Thr Giy Gly She Phe Cys Cys Leu Val Gly Ser Asn Gin *** *** Asn Leu Gly Gln Val
Pro Pro Leu Thr Gly Pro Thr Thr Pro Ser Pro Ser Pro *** Pro Ile Ala Pro GIn Pro Tyr Leu Val Pro Leu pro Leu Leu Leu Ala pro Asn His Tyr Pro pro Lys Pro thr Phe Tyr Arg Ser His gyr Ser Phe Pro gin Thr Ile Thr Hes Arg AAA CCC CCA TTT CAI GGC CCT CAC CAT CCT CTT CCC GAC CCA ATA CCA TAC CGC 1260 CO 1296 TTT GGE GGT AAA GTA CCG GGA GIG GTA GGA GAA GGG CTG GGT TAT GGT ATG GCG Phe Gly Gly Lys Val Pro Gly Val Val Gly Glu Gly Ieu Gly Tyr Gly Met Ala Leu Gly Val Lys Tyr Arg Giu Trp *xx Glu Lys Giy Tro Val Met Val $\operatorname{Trp}$ Arg $\operatorname{Trp}$ Gly *** Ser Thr Gly Ser Gly Arg Arg Arg Ala Gly Leu Trp Tyr Gly Gly

Pro Thr Thr *** Met Pro Thr Met Pro Ser Pro Gln Pro Arg Gln *** Leu Thr Leu Leu Leu Lys Cys Ieu Pro *** Leu His Pro Ser His Gly Lys Asn Cys Leu Ser Ser Tyr Asn Val Tyr Pro Asp gyr Thr Leu Ala Thr Alà Lys Thr Val phe COT CCT CAT CAA ATG TAT CCC CAG TAT CCA CTC CCG ACA CCG GAA ACA ATG , TTP gGa gga gia git tac ata gga gic aia get gag gac tgr gac cit tgT tac ana
Gly Gly Val Val Tyr Ile Gly Val Ile Gly Glu Gly Cys Gly Leu Cys Tyr Lys Glu Giu *** Phe Thr *** Gly Ser **x Val Arg Ala Val Ala Phe Val Thy ys Arg Ser Ser Jeu His Arg G-y His Arg *** GLy Ieu Trp Pro Leu Leu Gln Ser
${ }_{* * x}$ Ile Met **** Phe Leu Leu Val Pro Ala Trp Glu Gly Thr Val Arg Pro Ser Arg
******* Arg Phe Tyr Cys Cys G_n Leu Gly Ser Gly Gin *** Gly Pro His Asp Asn Asp Asp Leu Ile Val Ala Ser Ser Gly Val Gly Arg Asp Gly Gin Thr -le CAA TAG TAG ATT TTA TTG TCG IGA CCT CGG ETG , AGG GGA CAG TGG GAC CCA CTA GTT ATC ATC TAA AAT AAC AGC AC工 GGA GCC CAC TCC CCT GTC ACC CTG GGT GAT Val Tle Ile *** Asn Asn Ser Thr Gly Ala His Ser Pro Val Thr Leu Gly Asp Leu Ser Ser Lys Ile Thr Ala Leu Giu Pro Thr Pro Leu Ser Pro Trp Val Tle Tyr His Jeu Lys *** GIn His Trp Ser Pro Leu Pro Cys His Pro Gly *** Ser
Pro Ala Pro Gly Ser Asn Leu Arg Leu Arg Glu *** Glu Thr Thr Asn Leu Pro Pro Leu Leu Ala Leu Ile *** G-y*** Gly Lys Lys Asn Gln Leu Ile *** Leu. Pro Ser Cys Pro Trp Phe Glu Va_ Lys Val Lys Arg Ile Arg Tyr Tyr Glu Phe GCC CCI CGT CCC GET CTT AAG ITG GAA TTG GAA AGA ATA AGA CAT CAT AAG TTT CGE GGA GCA GGG CCA GAA TTC AAC CTT AAC CTT TC工 TAT TCT GTA GIA TTC AAA Arg Gly Ala Gly pro Glu Phe Asn Leu Asn Leu Ser Tyr Ser Val Val Pie Lys Gly Gu Gin Giy Gln Asn Ser Thr Leu Thr The Leu Ile Leu *** Tyr Ser Lys Gly Ser Arg Ala Arg Ile Gin Pro *** Pro Phe Leu Phe Cys Ser Ile Gin Arg
Cys Leu Ala Pro Thr Gln Gly Gly Glu Gln Pro Phe Phe Thr Net Iev Ile Ser Pro Val Ser Arg Pro Asn Ser gly Gly gly Pro Pro jeu Phe Aso Asn Ilè Asn CCC GTG TCT CGC CCC CAA ACT GGG GGG AGG ACC CCC TTC TTY CAG TAA TTA TAA GGE CAC 1467 ACA GCG GGG GTP TGA CCC 1485 CCC TCC IGG 1494 GGG AAA GTC ATT AAT ATT Gly His Arg Ala Gly Val *** Pro Pro Ser Tro Gly yys Lys Val Ile Asn -ie Gly Thr Glu Arg Giy Phe Asp Pro Pro Pro Gly Gy Arg Lys Ser Leu Tle Leu
Asp ****** Thr Trp Arg Gly Pro Pro Arg Glu Ser Gln Pro Glu Ser Ser Leu
Ile Glu Asp His Gly Gly Gly Leu Leu Ala Asn Gin Ser His Asn Ala Gln Cys Phe Arg Met Met Asp Val-Ala Trp Ser Pro Thr Arg Val Thr Thr Arg Lys Val CCT AGA GTA GTA CAG GTG GCG GGח CCT CCC ECA AGA CTG ACA CCA AGC GRA CTG
 GAA ICI CAT CAT GTC CAC CGC CCA GGA GGG CGI TCI GAC TGT GGT TCG CTT GAC Glu Ser His His Val His Aro Pro Gly Gly Arg Ser Aso Cys Gly Ser Leu Asp Asn Leu Tle Met Ser Thr Ala Gn Giu Gly Val Leu Thr Val Val Arg Leu Thr Ile Ser Ser Cys Pro Pro Pro Arg Arg Ala Phe *** Leu Tro Phe Ala **x Gln
Ile Asp Ser Pro Ala Pro Ser Ala Pro Thr Ser Ser Ala Met Lys Gly Glu Gly Tyr Ile Arg Leu His Pro Leu Pro Bro His Gln Teu His Tre Lys Glu Lys Glu Thr Tyr Gly Phe Thr Arg Ser Leu Arg Thr Asn Phe Ile Gly Asn Lys Arg Arg TCA TAT AGG CTT CCA CGC CCT CTC CGC CCA CAA $15 \square$ CTA CGG TAA AAA GGA AGA AGT ATA TCC GAA GGT GCG GGA GAG GCG GGR GTT GAA GAT GCC ATT TTT CTT -CT -- --. --
Ser Ile Ser Glu Gly Ala Gly glu Ala Gly Val glu Aso Ala Ile Phe Pro Ser
Val Tyr Pro Lys Val Arg Glu Arg Arg Val Leu Iys Met Pro Phe Phe Leu Leu Tyr fle Arg Arg Cys Gly Arg Gly Gly Cys *** Arg Cys His Phe Ser Phe Ser
Ala Thr Val Thr Ala Fro Thr Ser Ser Gly Pro Ala Ala Ala Ser Ser Arg Ala Leu Pro Leu Pro Pro Pro Pro Pro Arg Ala Leu Pro Pro Pro Pro Pro Asp Pro Trp Arg Tyr Arg His Arg Pro His Val Leu Trp Pro Arg Arg Arg Leu Ile Gin
GGI CGC CAT TGC CAC CGC CCC CAC CTG CTC GGT CCC CGC CGC CGC CTC CTA GAC 16298165 CCA GCG GTA ACG GIG GCG GGG GIG GAC GAG CCA GEG GCG GCG GCG GAG GAT CIG Fro Ala Val Thr Val Ala gly Val Asp Glu Pro Gly Ala Ala Ala Glu Asp Leu Gln Arg *** Arg Trp Arg Gly Trp Thr Ser Gln Gly Arg Arg Arg Arg Ile Tro Ser Gly Asn Gly Gly Gly Gly Gly Arg Ala Arg Gly Gly Gly Gly Gly Ser Gly
Leu Ile Ala Ala Pro Ala Thr Asp Glu Glu Glu Thr Val Gly Gly Gln Ile Arg
Trp Ser Pro Gln Pro Pro Pro Thr Lys Lys Lys Pro Leu Ala Glu Lys Ser Val
Gly Leu His Ser Arg Pro Arg His Arg Arg Arg Arg Tyr Arg Arg Arg Pro Iyr
CGG TTC 168 TAC CGA CGC CCC CGC CAC 1692 AGA AGA AGA AGC CAT TGC GGA GGA ACC IAT 1710
GCC AAG ATG GCT GCG GGG GCG GIG TCI TCT TCT TCG GTA ACG CCT CCT TGG ATA
Ala Iys Met Ala Ala Gly Ala Val Ser Ser Ser Ser Val Thr Pro Pro Thr Ile
Pro Arg Trp Leu Arg Gly Aro Cys Leu Leu Leu Arg *** Arg Leu Leu Gly Cyr
Gln Asp gly Cys Gly Gly Gly Val Phe Phe Phe Gly Asn Ala Ser Leu Asp thr
*** Ile Gln Phe Arg Phe Phe His Ala Thr Leu Ile
Asp Tyr Arg Phe Val Phe Ser Thr Arg Gln Leu Tyr
Thr Met Asp Ser Phe Ser Leu Leu Ala Ser Tyr Thr Asn
gCA gTA TAG ACT TTT GCT TTC TTC ACG CGA CAT TCA TAA 5'
CGT CAT ATC TGA AAA CGA AAG AAG TGC GCT GTA AGT ATT $3^{\prime}$
--- --- --- --- --- --- --- --- --- --- --- --- ---
Arg His Ile *** Lys Arg Lys Lys Cys Ala Val Ser Ile
Val Ile Ser Glu Asn glu Aro Ser Ala Leu *** Val
Ser Tyr Leu Lys Thr Lys Giu Val Arg Cys Lys Tyr

FIG. 8g
DMG IN GRAMS

FIG. 9

FIG. 10

FIG. 11

FIG. 12

FIG. 13

FIG. 14



## CIRCOVIRUS SEQUENCES ASSOCIATED WITH PIGLET WEIGHT LOSS DISEASE (PWD)

## INFORMATION ON RELATED APPLICATIONS

[0001] This application is a continuation of U.S. patent application Ser. No. 11/588,237, filed Oct. 27, 2006, now U.S. Pat. No. $\qquad$ , which is a divisional of U.S. patent application Ser. No. 10/718,264, filed Nov. 21, 2003, now U.S. Pat. No. $7,179,472$, which is a divisional of U.S. patent application Ser. No. 09/514,245, filed Feb. 28, 2000, now U.S. Pat. No. 6,703,023, which is a continuation-in-part of International Patent Application No. PCT/FR98/02634, filed Dec. 4, 1998, published in a non-English language, and now abandoned, which claims priority to French Application No. 97/15396, filed Dec. 5, 1997, the specifications of which are incorporated herein by reference in their entireties for all purposes.

## BACKGROUND OF THE INVENTION

[0002] The invention relates to the genomic sequence and nucleotide sequences coding for polypeptides of PWD circovirus, such as the structural and nonstructural polypeptides of said circovirus, as well as vectors including said sequences and cells or animals transformed by these vectors. The invention likewise relates to methods for detecting these nucleic acids or polypeptides and kits for diagnosing infection by the PWD circovirus. The invention is also directed to a method for selecting compounds capable of modulating the viral infection. The invention further comprises pharmaceutical compositions, including vaccines, for the prevention and/or the treatment of viral infections by PWD circovirus as well as the use of a vector according to the invention for the prevention and/or the treatment of diseases by gene therapy.
[0003] Piglet weight loss disease (PWD), alternatively called fatal piglet wasting (FPW) has been widely described in North America (Harding, J. C., 1997), and authors have reported the existence of a relationship between this pathology and the presence of porcine circovirus (Daft, B. et al., 1996; Clark, E. G., 1997; Harding, J. C., 1997; Harding, J. C. and Clark, E. G., 1997; Nayar, G. P. et al., 1997). A porcine circovirus has already been demonstrated in established lines of cell cultures derived from pigs and chronically infected (Tischer, I., 1986, 1988, 1995; Dulac, G. C., 1989; Edwards, S., 1994; Allan, G. M., 1995 and McNeilly, F., 1996). This virus, during experimental infection of piglets, does not prove pathogenic for pigs (Tischer, I., 1986, Homer, G. W., 1991) and its nucleotide sequence has been determined and characterized (Tischer, I., 1982; Meehan, B. M. et al., 1997; Mankertz., A., 1997). The porcine circovirus, called PCV virus, is part of the circovirus genus of the circoviridae family (Murphy, F.A. et al., 1995) whose virion has a circular DNA of size between 1.7 and 2.3 kb , which DNA comprises three open reading frames (ORF1 to ORF3), coding for a replication protein REP involved in the initiation and termination phase of rolling circular replication (RCR) (Heyraud-Nitschke, F., et al., 1995; Harding, M. R. et al., 1993; Hanson, S. F. et al., 1995; Fontes, E. P. B. et al., 1994), coding for a capsid protein (Boulton, L. H. et al., 1997; Hackland, A. F. et al., 1994; Chu, P. W. G. et al., 1993) and coding for a nonstructural protein called a dissemination protein (Lazarowitz., S. G. et al., 1989)
[0004] The inventors of the present invention have noticed that the clinical signs perceptible in pigs and linked to infection by the PWD circovirus are very distinctive. These manifestations in general appear in pigs of 8 to 12 weeks of age, weaned for 4 to 8 weeks. The first signs are hypotonia without it being possible to speak of prostration. Rapidly ( 48 hours), the flanks hollow, the line of the spine becomes apparent, and the pigs "blanch." These signs are in general accompanied by hyperthermia, anorexia and most often by respiratory signs (coughing, dyspnea, polypnea). Transitory diarrhea can likewise appear. The disease state phase lasts approximately one month at the end of which the rate of mortality varies from 5 to $20 \%$. To these mortalities, it is expedient to add a variable proportion (5-10\%) of cadaveric animals which are no longer able to present an economic future. It is to be noted that outside of this critical stage of the end of post-weaning, no anomaly appears on the farms. In particular, the reproductive function is totally maintained.
[0005] On the epidemiological level, the first signs of this pathology appeared at the start of 1995 in the east of the Côtes d'Armor region in France, and the farms affected are especially confined to this area of the region. In December 1996, the number of farms concerned could not be evaluated with precision because of the absence of a specific laboratory diagnostic method or of an epidemiological surveillance system of the livestock. Based on the clinical facts as well as on results of postmortem examinations supplied by veterinarians, it is possible to estimate this number as several dozen ( $80-100$ ). The contagiousness of the disease is weak to moderate. Cases are being reported outside the initial area and for the majority are following the transfer of animals coming from farms familiar with the problem. On the other hand, a characteristic of the condition is its strong remanence. Thus, farms which have been affected for a year are still affected in spite of the massive administration of therapeutics. Farms with clinical expression are drawn from various categories of specialization (breeders/fatteners, post-weaners/ fatteners) and different economic structures are concerned. In addition, the disorders appear even in farms where the rules of animal husbandry are respected.
[0006] Numerous postmortem examinations have been carried out either on farms or in the laboratory. The elements of the lesional table are disparate. The most constant macroscopic lesions are pneumonia which sometimes appears in patchy form as well as hypertrophy of the lymphatic ganglia. The other lesions above all affect the thoracic viscera including, especially, pericarditis and pleurisy. However, arthritis and gastric ulcers are also observed. The lesions revealed in the histological examination are essentially situated at the pulmonary level (interstitial pneumonia), ganglionic level (lymphoid depletion of the lymph nodes, giant cells) and renal level (glomerulonephritis, vasculitis). The infectious agents have been the subject of wide research. It has been possible to exclude the intervention of pestiviruses and Aujeszky's disease. The disorders appear in the seropositive PDRS (Porcine Dysgenic and Respiratory Syndrome, an infection linked to an arteriovirus) herds, but it has not been possible to establish the role of the latter in the genesis of the disorders (the majority of the farms in Brittany are PDRS seropositive).
[0007] The inventors of the present invention, with the aim of identifying the etiological agent responsible for PWD, have carried out "contact" tests between piglets which are obviously "ill" and SPF pigs (specific pathogen-free) from

CNEVA (Centre National d'Etudes Vétérinaires et Alimentaires, France). These tests allow the development of signs comparable to those observed on the farm to be observed in protected animal houses. The discrete signs such as moderate hyperthermia, anorexia and intermittent diarrhea appeared after one week of contact. It must be noted that the PDRS virus only diffused subsequent to the clinical signs. In addition, inoculations of organ homogenates of sick animals to healthy pigs allowed signs related to those observed on the farms to be reproduced, although with a lower incidence, linked to the favorable conditions of upkeep of the animals in the experimental installations.
[0008] Thus, the inventors of the present invention have been able to demonstrate that the pathological signs appear as a well-defined entity affecting the pig at a particular stage of its growth.
[0009] This pathology has never been described in France. However, sparse information, especially Canadian, relates to similar facts.
[0010] The disorders cannot be mastered with the existing therapeutics.
[0011] The data collected both on the farm and by experimentation have allowed the following points to be highlighted:
[0012] PWD is transmissible but its contagiousness is not very high,
[0013] its etiological origin is of infectious and probably viral nature,
[0014] PWD has a persistent character in the affected farms.
[0015] Considerable economic consequences ensue for the farms.
[0016] Thus, there is currently a significant need for a specific and sensitive diagnostic, whose production is practical and rapid, allowing the early detection of the infection.
[0017] A reliable, sensitive and practical test which allows the distinction between strains of porcine circovirus (PCV) is thus strongly desirable.
[0018] On the other hand, a need for efficient and welltolerated treatment of infections with PWD circovirus likewise remains desirable, no vaccine currently being available against PWD circovirus.
[0019] Concerning PWD circovirus, it will probably be necessary to understand the role of the immune defense in the physiology and the pathology of the disease to develop satisfactory vaccines.
[0020] Fuller information concerning the biology of these strains, their interactions with their hosts, the associated infectivity phenomena and those of escape from the immune defenses of the host especially, and finally their implication in the development of associated pathologies, will allow a better understanding of these mechanisms. Taking into account the facts which have been mentioned above and which show in particular the limitations of combating infection by the PWD circovirus, it is thus essential today on the one hand to develop molecular tools, especially starting from a better genetic knowledge of the PWD circovirus, and likewise to perfect novel preventive and therapeutic treatments, novel methods of diagnosis and specific, efficacious and tolerated novel vaccine strategies. This is precisely the subject of the present invention.

## SUMMARY OF THE INVENTION

[0021] The present invention relates to vaccines comprising a nucleotide sequence of the genome of Porcine circovirus
type B , or a homologue or fragment thereof, and an acceptable pharmaceutical or veterinary vehicle. In one embodiment of the invention, the nucleotide sequence is selected from SEQ ID No. 15, SEQ ID No. 19 SEQ ID No. 23, or SEQ ID No. 25, or a homologue or fragment thereof. In another embodiment of the invention, the homologue has at least $80 \%$ sequence identity to SEQ ID No. 15, SEQ ID No. 19, SEQ ID No. 23 or SEQ ID No. 25. In yet another embodiment, the vaccines further comprising an adjuvant
[0022] The present invention also relates to vaccines comprising a polypeptide encoded by a nucleotide sequence of the genome of PCVB, or a homologue or fragment thereof, and an acceptable pharmaceutical or veterinary vehicle. In one embodiment, the homologue has at least $80 \%$ sequence identity to SEQ ID No. 15, SEQ ID No. 19, SEQ ID No. 23 or SEQ ID No. 25. In another embodiment of the invention, the nucleotide sequence is selected from SEQ ID No. 23 or SEQ ID No. 25 , or a homologue or fragment thereof. In still another embodiment, the polypeptide has the amino acid sequence of SEQ ID No. 24 or SEQ ID No. 26. In yet another embodiment, the homologue has at least $80 \%$ sequence identity to SEQ ID No. 24 or SEQ ID No. 26. In another embodiment, the polypeptide has the amino acid sequence of SEQ ID No. 29, SEQ ID No. 30, SEQ ID No. 31, or SEQ ID No. 32.
[0023] A further aspect of the invention relates to vaccines comprising a vector and an acceptable pharmaceutical or veterinary vehicle, the vector comprising a nucleotide sequence of the genome of Porcine circovirus type $B$, or a homologue or fragment thereof. In one embodiment, the vaccine further comprises a gene coding for an expression product capable of inhibiting or retarding the establishment or development of a genetic or acquired disease.
[0024] The present invention also relates to vaccines comprising a cell and an acceptable pharmaceutical or veterinary vehicle, wherein the cell is transformed with a nucleotide sequence of the genome of Porcine circovirus type $B$, or a homologue or fragment thereof.
[0025] Still further, the present invention relates to vaccines comprising a pharmaceutically acceptable vehicle and a single polypeptide, wherein the single polypeptide consists of SEQ ID No. 26.
[0026] Additionally, the present invention relates to methods of immunizing a mammal against piglet weight loss disease comprising administering to a mammal an effective amount of the vaccines described above.
[0027] These and other aspects of the invention will become apparent to the skilled artisan in view of the teachings contained herein.

## BRIEF DESCRIPTION OF THE DRAWINGS

[0028] FIG. 1: Experimental scheme which has made it possible to bring about the isolation and the identification of the circovirus associated with PWD of type A and B.
[0029] Test 1: experimental reproduction of the PWD by inoculation of pig organ homogenates from farms affected by PWD.
[0030] Test 2: experimental reproduction of PWD.
[0031] Test 3: experimental reproduction of PWD.
[0032] Test 4: no experimental reproduction of PWD.
[0033] FIG. 2: Organization of the genome of the circovirus associated with PWD of type A (PCVA)
[0034] strand of (+) polarity (SEQ ID No. 1);
[0035] strand of (-) polarity (SEQ ID No. 5, represented according to the orientation $3^{\prime} \rightarrow 5^{\prime}$ );
[0036] sequences of amino acids of proteins encoded by the two DNA strands in the three possible reading frames SEQ ID NOS: 2-4 and 6-8 respectively.
[0037] FIG. 3: Alignment of the nucleotide sequence SEQ ID No. 1 of the PWD circovirus of type A (PCVA) and of the MEEHAN SEQ ID No. 163 strain and MANKERTZ SEQ ID No. 164 strain circoviruses of the porcine cell lines.
[0038] FIG. 4: Alignment of the sequence of amino acids SEQ ID No. 10 of a polypeptide encoded by the nucleotide sequence SEQ ID No. 9 (ORF1) of the PWD circovirus of type A (PCVA) and of corresponding nucleotide sequences of the MEEHAN SEQ ID No. 165 strain and MANKERTZ SEQ ID No. 166 strain circoviruses of the porcine cell lines.
[0039] FIG. 5: Alignment of the sequence of amino acids SEQ ID No. 12 of a polypeptide encoded by the nucleotide sequence SEQ ID No. 11 (ORF2) of the PWD circovirus of type A (PCVA) and of corresponding nucleotide sequences of the MEEHAN SEQ ID No. 167 strain and MANKERTZ SEQ ID No. 168 strain circoviruses of the porcine cell lines.
[0040] FIG. 6: Alignment of the sequence of amino acids SEQ ID No. 14 of a polypeptide encoded by the nucleotide sequence SEQ ID No. 13 (ORF3) of the PWD circovirus of type A (PCVA) and of corresponding nucleotide sequences of the MEEHAN SEQ ID No. 169 strain and MANKERTZ SEQ ID No. 170 strain circoviruses of the porcine cell lines.
[0041] FIG. 7: Western blot analysis of recombinant proteins of the PWD circovirus of type A (PCVA).
[0042] The analyses were carried out on cell extracts of Sf9 cells obtained after infection with recombinant baculovirus PCF ORF 1.
[0043] FIG. 8: Organization of the genome of the circovirus associated with the PWD of type B (PCVB)
[0044] strand of (+) polarity (SEQ ID No. 15);
[0045] strand of (-) polarity (SEQ ID No. 19, represented according to the orientation $3^{\prime} \rightarrow 5^{\prime}$ );
[0046] sequence of amino acids of proteins encoded by the two DNA strands in the three possible reading frames SEQ ID NOS: 16-18 and 20-22 respectively.
[0047] FIG. 9: Evolution of the daily mean gain (DMG) of pig farms affected by piglet weight loss disease (PWD), placed under experimental conditions.
[0048] FIG. 10: DMG compared for the 3 batches of pigs (F1, F3 and F4) calculated over a period of 28 days, after vaccination test.
[0049] FIG. 11: Hyperthermia greater than $41^{\circ}$ C., expressed as a percentage compared for the 3 batches of pigs (F1, F3 and F4) calculated per week over a period of 28 days, after vaccination test.
[0050] FIG. 12: Membranes of peptide spots corresponding to the ORF2s revealed with the aid of an infected pig serum, originating from a conventional farm.
[0051] The numbers of specific peptides of the circovirus of type B as well as their nonreactive homologs (type A) are indicated in bold.
[0052] The nonspecific immunogenic peptides are indicated in italics.
[0053] FIG. 13: Alignment of amino acid sequences of proteins encoded by the ORF2 of the PWD circovirus of type A SEQ ID No. 12 and by the ORF' 2 of the PWD circovirus of type B SEQ ID No. 26. The position of 4 peptides corresponding to specific epitopes of the PWD circovirus of type B is indicated on the corresponding sequence by a bold line, their homolog on the sequence of the PWD circovirus of type $A$ is likewise indicated by an ordinary line.
[0054] FIG. 14: Charts the results of experiments that demonstrate, in terms of percent hyperthermia, that vaccination with ORF' 1 and ORF' 2 of PCV-B enhances the level of protection in swine challenged with PCV-B (Percent hyperthermia: $>40.5 \mathrm{C}$, control: not vaccinated and not challenged, ORF'1: vaccinated and challenged, ORF'2: vaccinated and challenged, ORF: not vaccinated, challenged).
[0055] FIG. 15: Charts the results of experiments that demonstrate, in terms of animal growth, that vaccination with ORF'1 and ORF' 2 of PCV-B enhances the level of protection in swine challenged with PCV-B (Control: not vaccinated, not challenged, ORF'1: vaccinated and challenged, ORF'2: vaccinated and challenged, ORF: not vaccinated, challenged).
[0056] FIG. 16: Immunoperoxidase staining of PK15 cells at 24 h post-transfection with the pcDNA3/ORF'2 plasmid. Expression of PCVB ORF'2 was confirmed by IPMA following incubation in the presence of the swine anti-PCVB monospecific serum.

## DETAILED DESCRIPTION OF THE INVENTION

[0057] The present invention relates to nucleotide sequences of the genome of PWD circovirus selected from the sequences SEQ ID No. 1, SEQ ID No. 5, SEQ ID No. 15, SEQ ID No. 19 or one of their fragments.
[0058] The nucleotide sequences of sequences SEQ ID No. 1 and SEQ ID No. 5 correspond respectively to the genome sequence of the strand of $(+)$ polarity and of the strand of $(-)$ polarity of the PWD circovirus of type A (or PCVA), the sequence SEQ ID No. 5 being represented according to the orientation $5^{\prime} \rightarrow 3^{\prime}$.
[0059] The nucleotide sequences of sequences SEQ ID No. 15 and SEQ ID No. 19 correspond respectively to the genome sequence of the strand of $(+)$ polarity and of the strand of $(-)$ polarity of the PWD circovirus of type B (or PCVB), the sequence SEQ ID No. 19 being represented according to the orientation $5^{\prime} \rightarrow 3^{\prime}$.
[0060] The present invention likewise relates to nucleotide sequences, characterized in that they are selected from:
[0061] a) a nucleotide sequence of a specific fragment of the sequence SEQ ID No. 1, SEQ ID No. 5, SEQ ID No. 15, SEQ ID No. 19 or one of their fragments;
[0062] b) a nucleotide sequence homologous to a nucleotide sequence such as defined in a);
[0063] c) a nucleotide sequence complementary to a nucleotide sequence such as defined in a) or b), and a nucleotide sequence of their corresponding RNA;
[0064] d) a nucleotide sequence capable of hybridizing under stringent conditions with a sequence such as defined in a), b) or c);
[0065] e) a nucleotide sequence comprising a sequence such as defined in a), b), c) or d); and
[0066] f) a nucleotide sequence modified by a nucleotide sequence such as defined in a), b), c), d) or e).
[0067] Nucleotide, polynucleotide or nucleic acid sequence will be understood according to the present invention as meaning both a double-stranded or single-stranded DNA in the monomeric and dimeric (so-called in tandem) forms and the transcription products of said DNAs.
[0068] It must be understood that the present invention does not relate to the genomic nucleotide sequences taken in their natural environment, that is to say in the natural state. It concerns sequences which it has been possible to isolate, purify or partially purify, starting from separation methods such as, for example, ion-exchange chromatography, by
exclusion based on molecular size, or by affinity, or alternatively fractionation techniques based on solubility in different solvents, or starting from methods of genetic engineering such as amplification, cloning and subcloning, it being possible for the sequences of the invention to be carried by vectors.
[0069] The nucleotide sequences SEQ ID No. 1 and SEQ ID No. 15 were obtained by sequencing of the genome by the Sanger method.
[0070] Nucleotide sequence fragment according to the invention will be understood as designating any nucleotide fragment of the PWD circovirus, type A or B, of length of at least 8 nucleotides, preferably at least 12 nucleotides, and even more preferentially at least 20 consecutive nucleotides of the sequence from which it originates.
[0071] Specific fragment of a nucleotide sequence according to the invention will be understood as designating any nucleotide fragment of the PWD circovirus, type A or B, having, after alignment and comparison with the corresponding fragments of known porcine circoviruses, at least one nucleotide or base of different nature. For example, the specific nucleotide fragments of the PWD circovirus of type A can easily be determined by referring to FIG. 3 of the present invention in which the nucleotides or bases of the sequence SEQ ID No. 1 (circopordfp) are shown which are of different nature, after alignment of said sequence SEQ ID No. 1 with the other two sequences of known porcine circovirus (circopormeeh and circopormank).
[0072] Homologous nucleotide sequence in the sense of the present invention is understood as meaning a nucleotide sequence having at least a percentage identity with the bases of a nucleotide sequence according to the invention of at least $80 \%$, preferably $90 \%$ or $95 \%$, this percentage being purely statistical and it being possible to distribute the differences between the two nucleotide sequences at random and over the whole of their length.
[0073] Specific homologous nucleotide sequence in the sense of the present invention is understood as meaning a homologous nucleotide sequence having at least one nucleotide sequence of a specific fragment, such as defined above. Said "specific" homologous sequences can comprise, for example, the sequences corresponding to the genomic sequence or to the sequences of its fragments representative of variants of PWD circovirus of type A or B. These specific homologous sequences can thus correspond to variations linked to mutations within strains of PWD circovirus of type $A$ and $B$, and especially correspond to truncations, substitutions, deletions and/or additions of at least one nucleotide. Said homologous sequences can likewise correspond to variations linked to the degeneracy of the genetic code.
[0074] The term "degree or percentage of sequence homology" refers to "degree or percentage of sequence identity between two sequences after optimal alignment" as defined in the present application.
[0075] Two amino-acids or nucleotidic sequences are said to be "identical" if the sequence of amino-acids or nucleotidic residues, in the two sequences is the same when aligned for maximum correspondence as described below. Sequence comparisons between two (or more) peptides or polynucleotides are typically performed by comparing sequences of two optimally aligned sequences over a segment or "comparison window" to identify and compare local regions of sequence similarity. Optimal alignment of sequences for comparison may be conducted by the local homology algo-
rithm of Smith and Waterman, Ad. App. Math 2: 482 (1981), by the homology alignment algorithm of Neddleman and Wunsch, J. Mol. Biol. 48: 443 (1970), by the search for similarity method of Pearson and Lipman, Proc. Natl. Acad. Sci. (U.S.A.) 85: 2444 (1988), by computerized implementation of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group (GCG), 575 Science Dr., Madison, Wis.), or by visual inspection.
[0076] "Percentage of sequence identity" (or degree or identity) is determined by comparing two optimally aligned sequences over a comparison window, where the portion of the peptide or polynucleotide sequence in the comparison window may comprise additions or deletions (i.e., gaps) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical amino-acid residue or nucleic acid base occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison and multiplying the result by 100 to yield the percentage of sequence identity.
[0077] The definition of sequence identity given above is the definition that would use one of skill in the art. The definition by itself does not need the help of any algorithm, said algorithms being helpful only to achieve the optimal alignments of sequences, rather than the calculation of sequence identity.
[0078] From the definition given above, it follows that there is a well defined and only one value for the sequence identity between two compared sequences which value corresponds to the value obtained for the best or optimal alignment.
[0079] In the BLAST N or BLASTP "BLAST 2 sequence", software which is available in the web site http://www.ncbi. nlm.nih.gov/gorf/b12.html, and habitually used by the inventors and in general by the skilled man for comparing and determining the identity between two sequences, gap cost which depends on the sequence length to be compared is directly selected by the software (i.e. 11.2 for substitution matrix BLOSUM-62 for length $>85$ ).
[0080] In the present description, PWD circovirus will be understood as designating the circoviruses associated with piglet weight loss disease (PWD) of type A (PCVA) or type B (PCVB), defined below by their genomic sequence, as well as the circoviruses whose nucleic sequences are homologous to the sequences of PWD circoviruses of type A or B, such as in particular the circoviruses corresponding to variants of the type A or of the type B.
[0081] Complementary nucleotide sequence of a sequence of the invention is understood as meaning any DNA whose nucleotides are complementary to those of the sequence of the invention, and whose orientation is reversed (antiparallel sequence).
[0082] Hybridization under conditions of stringency with a nucleotide sequence according to the invention is understood as meaning a hybridization under conditions of temperature and ionic strength chosen in such a way that they allow the maintenance of the hybridization between two fragments of complementary DNA.
[0083] By way of illustration, conditions of great stringency of the hybridization step with the aim of defining the nucleotide fragments described above are advantageously the following.
[0084] The hybridization is carried out at a preferential temperature of $65^{\circ} \mathrm{C}$. in the presence of SSC buffer, $1 \times$ SSC corresponding to 0.15 M NaCl and 0.05 M Na citrate. The washing steps, for example, can be the following:
[0085] $2 \times$ SSC, at ambient temperature followed by two washes with $2 \times \operatorname{SSC}, 0.5 \% \mathrm{SDS}$ at $65^{\circ} \mathrm{C}$.; $2 \times 0.5 \times \mathrm{SSC}$, $0.5 \%$ SDS; at $65^{\circ} \mathrm{C}$. for 10 minutes each.
[0086] The conditions of intermediate stringency, using, for example, a temperature of $42^{\circ} \mathrm{C}$. in the presence of a $2 \times$ SSC buffer, or of less stringency, for example a temperature of $37^{\circ} \mathrm{C}$. in the presence of a $2 \times \mathrm{SSC}$ buffer, respectively require a globally less significant complementarity for the hybridization between the two sequences.
[0087] The stringent hybridization conditions described above for a polynucleotide with a size of approximately 350 bases will be adapted by the person skilled in the art for oligonucleotides of greater or smaller size, according to the teaching of Sambrook et al., 1989.
[0088] Among the nucleotide sequences according to the invention, those are likewise preferred which can be used as a primer or probe in methods allowing the homologous sequences according to the invention to be obtained, these methods, such as the polymerase chain reaction (PCR), nucleic acid cloning and sequencing, being well known to the person skilled in the art.
[0089] Among said nucleotide sequences according to the invention, those are again preferred which can be used as a primer or probe in methods allowing the presence of PWD circovirus or one of its variants such as defined below to be diagnosed.
[0090] The nucleotide sequences according to the invention capable of modulating, of inhibiting or of inducing the expression of PWD circovirus gene, and/or capable of modulating the replication cycle of PWD circovirus in the host cell and/or organism are likewise preferred. Replication cycle will be understood as designating the invasion and the multiplication of PWD circovirus, and its propagation from host cell to host cell in the host organism.
[0091] Among said nucleotide sequences according to the invention, those corresponding to open reading frames, called ORF sequences, and coding for polypeptides, such as, for example, the sequences SEQ ID No. 9 (ORF1), SEQ ID No. 11 (ORF2) and SEQ ID No. 13 (ORF3) respectively corresponding to the nucleotide sequences between the positions 47 and 985 determined with respect to the position of the nucleotides on the sequence SEQ ID No. 1, the positions 1723 and 1022 and the positions 658 and 38 with respect to the position of the nucleotides on the sequence SEQ ID No. 5 (represented according to the orientation $3^{\prime} \rightarrow 5^{\prime}$ ), the ends being included, or alternatively the sequences SEQ ID No. 23 (ORF'1), SEQ ID No. 25 (ORF'2) and SEQ ID No. 27 (ORF'3), respectively corresponding to the sequences between the positions 51 and 995 determined with respect to the position of the nucleotides on the sequence SEQ ID No. 15 , the positions 1734 and 1033 and the positions 670 and 357 , the positions being determined with respect to the position of the nucleotides on the sequence SEQ ID No. 19 (represented according to the orientation $3^{\prime} \rightarrow 5^{\prime}$ ), the ends being included, are finally preferred.
[0092] The nucleotide sequence fragments according to the invention can be obtained, for example, by specific amplification, such as PCR, or after digestion with appropriate restriction enzymes of nucleotide sequences according to the invention, these methods in particular being described in the
work of Sambrook et al., 1989. Said representative fragments can likewise be obtained by chemical synthesis when their size is not very large and according to methods well known to persons skilled in the art
[0093] Modified nucleotide sequence will be understood as meaning any nucleotide sequence obtained by mutagenesis according to techniques well known to the person skilled in the art, and containing modifications with respect to the normal sequences according to the invention, for example mutations in the regulatory and/or promoter sequences of polypeptide expression, especially leading to a modification of the rate of expression of said polypeptide or to a modulation of the replicative cycle.
[0094] Modified nucleotide sequence will likewise be understood as meaning any nucleotide sequence coding for a modified polypeptide such as defined below.
[0095] The present invention relates to nucleotide sequences of PWD circovirus according to the invention, characterized in that they are selected from the sequences SEQ ID No. 9, SEQ ID No. 11, SEQ ID No. 13, SEQ ID No. 23, SEQ ID No. 25 , SEQ ID No. 27 or one of their fragments.
[0096] The invention likewise relates to nucleotide sequences characterized in that they comprise a nucleotide sequence selected from:
[0097] a) a nucleotide sequence SEQ ID No. 9, SEQ ID No. 11, SEQ ID No. 13, SEQ ID No. 23, SEQ ID No. 25, SEQ ID No. 27 or one of their fragments;
[0098] b) a nucleotide sequence of a specific fragment of a sequence such as defined in a);
[0099] c) a homologous nucleotide sequence having at least $80 \%$ identity with a sequence such as defined in a) or b);
[0100] d) a complementary nucleotide sequence or sequence of RNA corresponding to a sequence such as defined in a), b) or c); and
[0101] e) a nucleotide sequence modified by a sequence such as defined in a), b), c) or d).
[0102] As far as homology with the nucleotide sequences SEQ ID No. 9, SEQ ID No. 11, SEQ ID No. 13, SEQ ID No. 23, SEQ ID No. 25, SEQ ID No. 27 or one of their fragments is concerned, the homologous, especially specific, sequences having a percentage identity with one of the sequences SEQ ID No. 9, SEQ ID No. 11, SEQ ID No. 13, SEQ ID No. 23, SEQ ID No. 25, SEQ ID No. 27 or one of their fragments of at least $80 \%$, preferably $90 \%$ or $95 \%$, are preferred. Said specific homologous sequences can comprise, for example, the sequences corresponding to the sequences ORF1, ORF2, ORF3, ORF'1, ORF'2 and ORF'3 of PWD circovirus variants of type A or of type B. In the same manner, these specific homologous sequences can correspond to variations linked to mutations within strains of PWD circovirus of type A or of type B and especially correspond to truncations, substitutions, deletions and/or additions of at least one nucleotide.
[0103] Among nucleotide sequences according to the invention, the sequence SEQ ID No. 23 which has a homology having more than $80 \%$ identity with the sequence SEQ ID No. 9 , as well as the sequence SEQ ID No. 25 , are especially preferred.
[0104] Preferably, the invention relates to the nucleotide sequences according to the invention, characterized in that they comprise a nucleotide sequence selected from the following sequences:

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a) SEQ ID No. \(33 \quad 1705^{\prime}\) TGTGGCGA \(3^{\prime}\);
b) SEQ ID No. \(344505^{\prime}\) AGTTTCCT \(3^{\prime}\);
c) SEQ ID No. \(3510265^{\prime}\) TCATTTAGAGGGTCTTTCAG \(3^{\prime}\);
d) SEQ ID No. \(3610745^{\prime}\) GTCAACCT \(3^{\prime} ;\)
e) SEQ ID No. \(3711015^{\prime}\) GTGGTTGC \(3^{\prime} ;\)
f) SEQ ID No. \(3811235^{\prime}\) AGCCCAGG \(3^{\prime \prime}\);
g) SEQ ID No. \(3911925^{\prime}\) ' TTGGCTGG \(3^{\prime}\);
h) SEQ ID No. \(4012185^{\prime}\) TCTAGCTCTGGT \(3^{\prime \prime} ;\)
i) SEQ ID No. 411501 5' ATCTCAGCTCGT 3';
j) \(\operatorname{SEQ}\) ID No. \(4215365^{\prime}\) TGTCCTCCTCTT \(3^{\prime}\);
k) SEQ ID No. \(4315635^{\prime}\) TCTCTAGA \(3^{\prime}\);
1) SEQ ID No. \(4416235^{\prime}\) TGTACCAA \(3^{\prime}\);
m) SEQ ID No. \(4516865^{\prime}\) TCCGTCTT 3';
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and their complementary sequences.
[0105] In the list of nucleotide sequences a)-m) above, the underlined nucleotides are mutated with respect to the two known sequences of circovirus which are nonpathogenic to pigs. The number preceding the nucleotide sequence represents the position of the first nucleotide of said sequence in the sequence SEQ ID No. 1.
[0106] The invention comprises the polypeptides encoded by a nucleotide sequence according to the invention, preferably a polypeptide whose sequence is represented by a fragment, especially a specific fragment, of one of the six sequences of amino acids represented in FIG. 2, these six amino acid sequences corresponding to the polypeptides which can be encoded according to one of the three possible reading frames of the sequence SEQ ID No. 1 or of the sequence SEQ ID No. 5 , or a polypeptide whose sequence is represented by a fragment, especially a specific fragment, of one of the six sequences of amino acids shown in FIG. 8, these six sequences of amino acids corresponding to the polypeptides which can be encoded according to one of the three possible reading frames of the sequence SEQ ID No. 15 or of the sequence SEQ ID No. 19.
[0107] The invention likewise relates to the polypeptides, characterized in that they comprise a polypeptide selected from the amino acid sequences SEQ ID No. 10, SEQ ID No. 12, SEQ ID No. 14, SEQ ID No. 24, SEQ ID No. 26, SEQ ID No. 28 or one of their fragments.
[0108] Among the polypeptides according to the invention, the polypeptide of amino acid sequence SEQ ID No. 24 which has a homology having more than $80 \%$ identity with the sequence SEQ ID No. 10 , as well as the polypeptide of sequence SEQ ID No. 26, are especially preferred.
[0109] The invention also relates to the polypeptides, characterized in that they comprise a polypeptide selected from:
[0110] a) a specific fragment of at least 5 amino acids of a polypeptide of an amino acid sequence according to the invention;
[0111] b) a polypeptide homologous to a polypeptide such as defined in a);
[0112] c) a specific biologically active fragment of a polypeptide such as defined in a) or b); and
[0113] d) a polypeptide modified by a polypeptide such as defined in $a$ ), b) or c).
[0114] Among the polypeptides according to the invention, the polypeptides of amino acid sequences SEQ ID No. 29, SEQ ID No. 30, SEQ ID No. 31 and SEQ ID No. 32 are also preferred, these polypeptides being especially capable of specifically recognizing the antibodies produced during infection by the PWD circovirus of type B. These polypeptides thus have epitopes specific for the PWD circovirus of type B and can thus be used in particular in the diagnostic field or as immunogenic agent to confer protection in pigs against infection by PWD circovirus, especially of type B.
[0115] In the present description, the terms polypeptide, peptide and protein are interchangeable.
[0116] It must be understood that the invention does not relate to the polypeptides in natural form, that is to say that they are not taken in their natural environment but that they can be isolated or obtained by purification from natural sources, or else obtained by genetic recombination, or alternatively by chemical synthesis and that they can thus contain unnatural amino acids, as will be described below.
[0117] Polypeptide fragment according to the invention is understood as designating a polypeptide containing at least 5 consecutive amino acids, preferably 10 consecutive amino acids or 15 consecutive amino acids.
[0118] In the present invention, specific polypeptide fragment is understood as designating the consecutive polypeptide fragment encoded by a specific fragment nucleotide sequence according to the invention.
[0119] Homologous polypeptide will be understood as designating the polypeptides having, with respect to the natural polypeptide, certain modifications such as, in particular, a deletion, addition or substitution of at least one amino acid, a truncation, a prolongation, a chimeric fusion, and/or a mutation. Among the homologous polypeptides, those are preferred whose amino acid sequence has at least $80 \%$, preferably $90 \%$, homology with the sequences of amino acids of polypeptides according to the invention.
[0120] Specific homologous polypeptide will be understood as designating the homologous polypeptides such as defined above and having a specific fragment of polypeptide according to the invention.
[0121] In the case of a substitution, one or more consecutive or nonconsecutive amino acids are replaced by "equivalent" amino acids. The expression "equivalent" amino acid is directed here at designating any amino acid capable of being substituted by one of the amino acids of the base structure without, however, essentially modifying the biological activities of the corresponding peptides and such that they will be defined by the following.
[0122] These equivalent amino acids can be determined either by depending on their structural homology with the amino acids which they substitute, or on results of comparative tests of biological activity between the different polypeptides, which are capable of being carried out.
[0123] By way of example, the possibilities of substitutions capable of being carried out without resulting in an extensive modification of the biological activity of the corresponding modified polypeptides will be mentioned, the replacement, for example, of leucine by valine or isoleucine, of aspartic acid by glutamic acid, of glutamine by asparagine, of arginine by lysine etc., the reverse substitutions naturally being envisageable under the same conditions.
[0124] The specific homologous polypeptides likewise correspond to polypeptides encoded by the specific homologous nucleotide sequences such as defined above and thus comprise in the present definition the polypeptides which are mutated or correspond to variants which can exist in PWD circovirus, and which especially correspond to truncations, substitutions, deletions and/or additions of at least one amino acid residue.
[0125] Specific biologically active fragment of a polypeptide according to the invention will be understood in particular as designating a specific polypeptide fragment, such as defined above, having at least one of the characteristics of polypeptides according to the invention, especially in that it is:
[0126] capable of inducing an immunogenic reaction directed against a PWD circovirus; and/or
[0127] capable of being recognized by a specific antibody of a polypeptide according to the invention; and/or
[0128] capable of linking to a polypeptide or to a nucleotide sequence of PWD circovirus; and/or
[0129] capable of exerting a physiological activity, even partial, such as, for example, a dissemination or structural (capsid) activity; and/or
[0130] capable of modulating, of inducing or of inhibiting the expression of PWD circovirus gene or one of its variants, and/or capable of modulating the replication cycle of PWD circovirus in the cell and/or the host organism.
[0131] The polypeptide fragments according to the invention can correspond to isolated or purified fragments naturally present in a PWD circovirus or correspond to fragments which can be obtained by cleavage of said polypeptide by a proteolytic enzyme, such as trypsin or chymotrypsin or collagenase, or by a chemical reagent, such as cyanogen bromide ( CNBr ) or alternatively by placing said polypeptide in a very acidic environment, for example at pH 2.5 . Such polypeptide fragments can likewise just as easily be prepared by chemical synthesis, from hosts transformed by an expression vector according to the invention containing a nucleic acid allowing the expression of said fragments, placed under the control of appropriate regulation and/or expression elements.
[0132] "Modified polypeptide" of a polypeptide according to the invention is understood as designating a polypeptide obtained by genetic recombination or by chemical synthesis as will be described below, having at least one modification with respect to the normal sequence. These modifications will especially be able to bear on amino acids at the origin of a specificity, of pathogenicity and/or of virulence, or at the origin of the structural conformation, and of the capacity of membrane insertion of the polypeptide according to the invention. It will thus be possible to create polypeptides of equivalent, increased or decreased activity, and of equivalent, narrower, or wider specificity. Among the modified polypep-
tides, it is necessary to mention the polypeptides in which up to 5 amino acids can be modified, truncated at the N - or C-terminal end, or even deleted or added.
[0133] As is indicated, the modifications of the polypeptide will especially have as objective:
[0134] to render it capable of modulating, of inhibiting or of inducing the expression of PWD circovirus gene and/ or capable of modulating the replication cycle of PWD circovirus in the cell and/or the host organism,
[0135] of allowing its incorporation into vaccine compositions,
[0136] of modifying its bioavailability as a compound for therapeutic use.
[0137] The methods allowing said modulations on eukaryotic or prokaryotic cells to be demonstrated are well known to the person skilled in the art. It is likewise well understood that it will be possible to use the nucleotide sequences coding for said modified polypeptides for said modulations, for example through vectors according to the invention and described below, in order, for example, to prevent or to treat the pathologies linked to the infection.
[0138] The preceding modified polypeptides can be obtained by using combinatorial chemistry, in which it is possible to systematically vary parts of the polypeptide before testing them on models, cell cultures or microorganisms for example, to select the compounds which are most active or have the properties sought.
[0139] Chemical synthesis likewise has the advantage of being able to use:
[0140] unnatural amino acids, or
[0141] nonpeptide bonds.
[0142] Thus, in order to improve the duration of life of the polypeptides according to the invention, it may be of interest to use unnatural amino acids, for example in D form, or else amino acid analogs, especially sulfur-containing forms, for example.
[0143] Finally, it will be possible to integrate the structure of the polypeptides according to the invention, its specific or modified homologous forms, into chemical structures of polypeptide type or others. Thus, it may be of interest to provide at the N - and C-terminal ends compounds not recognized by the proteases.
[0144] The nucleotide sequences coding for a polypeptide according to the invention are likewise part of the invention.
[0145] The invention likewise relates to nucleotide sequences utilizable as a primer or probe, characterized in that said sequences are selected from the nucleotide sequences according to the invention.
[0146] Among the pairs of nucleotide sequences utilizable as a pair of primers according to the invention, the pairs of primers selected from the following pairs are preferred:

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a) SEQ ID No. 46 5' GTG TGC TCG ACA TTG GTG TG 3', and
SEQ ID No. 47 5' TGG AAT GTT AAC GAG CTG AG 3';
b) SEQ ID NO. 46 5' GTG TGC TCG ACA TTG GTG TG 3', and
SEQ ID NO. 48 5' CTC GCA GCC ATC TTG GAA TG 3',
C) SEQ ID No. 49 5' CGC GCG TAA TAC GAC TCA CT 3', and
SEQ ID No. 46 5' GTG TGC TCG ACA TTG GTG TG 3';
d) SEQ ID NO. 49 5' CGC GCG TAA TAC GAC TCA CT 3', and
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-continued
SEQ ID No. 48 5' CTC GCA GCC ATC TTG GAA TG 3'; and
e) SEQ ID No. 50 5' CCT GTC TAC TGC TGT GAG TAC CTT GT 3', and
SEQ ID NO. 51 5' GCA GTA GAC AGG TCA CTC CGT TGT CC 3'.
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[0147] The cloning and the sequencing of the PWD circovirus, type A and B , has allowed it to be identified, after comparative analysis with the nucleotide sequences of other porcine circoviruses, that, among the sequences of fragments of these nucleic acids, were those which are strictly specific to the PWD circovirus of type A, of type B or of type A and B, and those which correspond to a consensus sequence of porcine circoviruses other than the PWD circoviruses of type A and/or B.
[0148] There is likewise a great need for nucleotide sequences utilizable as a primer or probe specific to the whole of the other known and nonpathogenic porcine circoviruses.
[0149] Said consensus nucleotide sequences specific to all circoviruses, other than PWD circovirus of type A and B, are easily identifiable from FIG. 3 and the sequence SEQ ID No. 15 , and are part of the invention.
[0150] Among said consensus nucleotide sequences, that which is characterized in that it is part of the following pair of primers is preferred:

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a) SEQ ID No. 46 5' GTG TGC TCG ACA TTG GTG TG 3',
    and
SEQ ID NO. 52 5' TGG AAT GTT AAC TAC CTC AA 3'.
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[0151] The invention likewise comprises a nucleotide sequence according to the invention, characterized in that said sequence is a specific consensus sequence of porcine circovirus other than PWD circovirus of type B and in that it is one of the primers of the following pairs of primers:

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a) SEQ ID No. 53 5' GGC GGC GCC ATC TGT AAC GGT
    TT 3', and
SEQ ID NO. 54 5' GAT GGC GCC GAA AGA CGG GTA
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[0152] It is well understood that the present invention likewise relates to specific polypeptides of known porcine circoviruses other than PWD circovirus, encoded by said consensus nucleotide sequences, capable of being obtained by purification from natural polypeptides, by genetic recombination or by chemical synthesis by procedures well known to the person skilled in the art and such as described in particular below. In the same manner, the labeled or unlabeled mono- or polyclonal antibodies directed against said specific polypeptides encoded by said consensus nucleotide sequences are also part of the invention.
[0153] It will be possible to use said consensus nucleotide sequences, said corresponding polypeptides as well as said antibodies directed against said polypeptides in procedures or sets for detection and/or identification such as described below, in place of or in addition to nucleotide sequences, polypeptides or antibodies according to the invention, specific to PWD circovirus type A and/or B.
[0154] These protocols have been improved for the differential detection of the circular monomeric forms of specific replicative forms of the virion or of the DNA in replication and the dimeric forms found in so-called in-tandem molecular constructs.
[0155] The invention additionally relates to the use of a nucleotide sequence according to the invention as a primer or probe for the detection and/or the amplification of nucleic acid sequences.
[0156] The nucleotide sequences according to the invention can thus be used to amplify nucleotide sequences, especially by the PCR technique (polymerase chain reaction) (Erlich, 1989; Innis et al., 1990; Rolfs et al., 1991; and White et al., 1997).
[0157] These oligodeoxyribonucleotide or oligoribonucleotide primers advantageously have a length of at least 8 nucleotides, preferably of at least 12 nucleotides, and even more preferentially at least 20 nucleotides.
[0158] Other amplification techniques of the target nucleic acid can be advantageously employed as alternatives to PCR.
[0159] The nucleotide sequences of the invention, in particular the primers according to the invention, can likewise be employed in other procedures of amplification of a target nucleic acid, such as:
[0160] the TAS technique (Transcription-based Amplification System), described by Kwoh et al. in 1989;
[0161] the 3SR technique (Self-Sustained Sequence Replication), described by Guatelli et al. in 1990;
[0162] the NASBA technique (Nucleic Acid Sequence Based Amplification), described by Kievitis et al. in 1991;
[0163] the SDA technique (Strand Displacement Amplification) (Walker et al., 1992);
[0164] the TMA technique (Transcription Mediated Amplification).
[0165] The polynucleotides of the invention can also be employed in techniques of amplification or of modification of the nucleic acid serving as a probe, such as:
[0166] the LCR technique (Ligase Chain Reaction), described by Landegren et al. in 1988 and improved by Barany et al. in 1991, which employs a thermostable ligase;
[0167] the RCR technique (Repair Chain Reaction), described by Segev in 1992;
[0168] the CPR technique (Cycling Probe Reaction), described by Duck et al. in 1990;
[0169] the amplification technique with Q-beta replicase, described by Miele et al. in 1983 and especially improved by Chu et al. in 1986, Lizardi et al. in 1988 , then by Burg et al. as well as by Stone et al. in 1996.
[0170] In the case where the target polynucleotide to be detected is possibly an RNA, for example an mRNA, it will be possible to use, prior to the employment of an amplification reaction with the aid of at least one primer according to the invention or to the employment of a detection procedure with the aid of at least one probe of the invention, an enzyme of reverse transcriptase type in order to obtain a cDNA from the RNA contained in the biological sample. The cDNA obtained will thus serve as a target for the primer(s) or the probe(s) employed in the amplification or detection procedure according to the invention.
[0171] The detection probe will be chosen in such a manner that it hybridizes with the target sequence or the amplicon generated from the target sequence. By way of sequence, such a probe will advantageously have a sequence of at least 12 nucleotides, in particular of at least 20 nucleotides, and preferably of at least 100 nucleotides.
[0172] The invention also comprises the nucleotide sequences utilizable as a probe or primer according to the invention, characterized in that they are labeled with a radioactive compound or with a nonradioactive compound.
[0173] The unlabeled nucleotide sequences can be used directly as probes or primers, although the sequences are generally labeled with a radioactive element $\left({ }^{32} \mathrm{P},{ }^{35} \mathrm{~S},{ }^{3} \mathrm{H}\right.$, ${ }^{125} \mathrm{I}$ ) or with a nonradioactive molecule (biotin, acetylaminofluorene, digoxigenin, 5-bromodeoxyuridine, fluorescein) to obtain probes which are utilizable for numerous applications.
[0174] Examples of nonradioactive labeling of nucleotide sequences are described, for example, in French Patent No. 78.10975 or by Urdea et al. or by Sanchez-Pescador et al. in 1988.
[0175] In the latter case, it will also be possible to use one of the labeling methods described in patents FR-2 422956 and FR-2 518755.
[0176] The hybridization technique can be carried out in various manners (Matthews et al., 1988). The most general method consists in immobilizing the nucleic acid extract of cells on a support (such as nitrocellulose, nylon, polystyrene) and in incubating, under well-defined conditions, the immobilized target nucleic acid with the probe. After hybridization, the excess of probe is eliminated and the hybrid molecules formed are detected by the appropriate method (measurement of the radioactivity, of the fluorescence or of the enzymatic activity linked to the probe).
[0177] The invention likewise comprises the nucleotide sequences according to the invention, characterized in that they are immobilized on a support, covalently or noncovalently.
[0178] According to another advantageous mode of employing nucleotide sequences according to the invention, the latter can be used immobilized on a support and can thus serve to capture, by specific hybridization, the target nucleic acid obtained from the biological sample to be tested. If necessary, the solid support is separated from the sample and the hybridization complex formed between said capture probe and the target nucleic acid is then detected with the aid of a second probe, a so-called detection probe, labeled with an easily detectable element.
[0179] Another subject of the present invention is a vector for the cloning and/or expression of a sequence, characterized in that it contains a nucleotide sequence according to the invention.
[0180] The vectors according to the invention, characterized in that they contain the elements allowing the expression and/or the secretion of said nucleotide sequences in a determined host cell, are likewise part of the invention.
[0181] The vector must then contain a promoter, signals of initiation and termination of translation, as well as appropriate regions of regulation of transcription. It must be able to be maintained stably in the host cell and can optionally have particular signals specifying the secretion of the translated protein. These different elements are chosen as a function of the host cell used. To this end, the nucleotide sequences
according to the invention can be inserted into autonomous replication vectors within the chosen host, or integrated vectors of the chosen host.
[0182] Such vectors will be prepared according to the methods currently used by the person skilled in the art, and it will be possible to introduce the clones resulting therefrom into an appropriate host by standard methods, such as, for example, lipofection, electroporation and thermal shock.
[0183] The vectors according to the invention are, for example, vectors of plasmid or viral origin.
[0184] A preferred vector for the expression of polypeptides of the invention is baculovirus.
[0185] The vector pBS KS in which is inserted the intandem DNA sequence of the PWD circovirus type A (or DFP) as deposited at the CNCM on 3 Jul. 1997, under the number I-1891, is likewise preferred.
[0186] These vectors are useful for transforming host cells in order to clone or to express the nucleotide sequences of the invention.
[0187] The invention likewise comprises the host cells transformed by a vector according to the invention.
[0188] These cells can be obtained by the introduction into host cells of a nucleotide sequence inserted into a vector such as defined above, then the culturing of said cells under conditions allowing the replication and/or expression of the transfected nucleotide sequence.
[0189] The host cell can be selected from prokaryotic or eukaryotic systems, such as, for example, bacterial cells (Olins and Lee, 1993), but likewise yeast cells (Buckholz, 1993), as well as animal cells, in particular the cultures of mammalian cells (Edwards and Aruffo, 1993), and especially Chinese hamster ovary (CHO) cells, but likewise the cells of insects in which it is possible to use procedures employing baculoviruses, for example (Luckow, 1993).
[0190] A preferred host cell for the expression of the proteins of the invention is constituted by sf 9 insect cells.
[0191] A more preferred host cell according to the invention is E. coli, such as deposited at the CNCM on 3 Jul. 1997, under the number I-1891.
[0192] The invention likewise relates to animals comprising one of said transformed cells according to the invention.
[0193] The obtainment of transgenic animals according to the invention overexpres sing one or more of the genes of PWD circovirus or part of the genes will be preferably carried out in rats, mice or rabbits according to methods well known to the person skilled in the art, such as by viral or nonviral transfections. It will be possible to obtain the transgenic animals overexpressing one or more of said genes by transfection of multiple copies of said genes under the control of a strong promoter of ubiquitous nature, or selective for one type of tissue. It will likewise be possible to obtain the transgenic animals by homologous recombination in embryonic cell strains, transfer of these cell strains to embryos, selection of the affected chimeras at the level of the reproductive lines, and growth of said chimeras.
[0194] The transformed cells as well as the transgenic animals according to the invention are utilizable in procedures for preparation of recombinant polypeptides.
[0195] It is today possible to produce recombinant polypeptides in relatively large quantity by genetic engineering using the cells transformed by expression vectors according to the invention or using transgenic animals according to the invention.
[0196] The procedures for preparation of a polypeptide of the invention in recombinant form, characterized in that they employ a vector and/or a cell transformed by a vector according to the invention and/or a transgenic animal comprising one of said transformed cells according to the invention, are themselves comprised in the present invention.
[0197] Among said procedures for preparation of a polypeptide of the invention in recombinant form, the preparation procedures employing a vector, and/or a cell transformed by said vector and/or a transgenic animal comprising one of said transformed cells, containing a nucleotide sequence according to the invention coding for a polypeptide of PWD circovirus, are preferred.
[0198] The recombinant polypeptides obtained as indicated above can just as well be present in glycosylated form as in nonglycosylated form and can or cannot have the natural tertiary structure.
[0199] A preferred variant consists in producing a recombinant polypeptide used to a "carrier" protein (chimeric protein). The advantage of this system is that it allows a stabilization of and a decrease in the proteolysis of the recombinant product, an increase in the solubility in the course of renaturation in vitro and/or a simplification of the purification when the fusion partner has an affinity for a specific ligand.
[0200] More particularly, the invention relates to a procedure for preparation of a polypeptide of the invention comprising the following steps:
[0201] a) culture of transformed cells under conditions allowing the expression of a recombinant polypeptide of nucleotide sequence according to the invention;
[0202] b) if need be, recovery of said recombinant polypeptide.
[0203] When the procedure for preparation of a polypeptide of the invention employs a transgenic animal according to the invention, the recombinant polypeptide is then extracted from said animal.
[0204] The invention also relates to a polypeptide which is capable of being obtained by a procedure of the invention such as described previously.
[0205] The invention also comprises a procedure for preparation of a synthetic polypeptide, characterized in that it uses a sequence of amino acids of polypeptides according to the invention.
[0206] The invention likewise relates to a synthetic polypeptide obtained by a procedure according to the invention.
[0207] The polypeptides according to the invention can likewise be prepared by techniques which are conventional in the field of the synthesis of peptides. This synthesis can be carried out in homogeneous solution or in solid phase.
[0208] For example, recourse can be made to the technique of synthesis in homogeneous solution described by HoubenWeyl in 1974.
[0209] This method of synthesis consists in successively condensing, two by two, the successive amino acids in the order required, or in condensing amino acids and fragments formed previously and already containing several amino acids in the appropriate order, or alternatively several fragments previously prepared in this way, it being understood that it will be necessary to protect beforehand all the reactive functions carried by these amino acids or fragments, with the exception of amine functions of one and carboxyls of the other or vice-versa, which must normally be involved in the
formation of peptide bonds, especially after activation of the carboxyl function, according to the methods well known in the synthesis of peptides.
[0210] According to another preferred technique of the invention, recourse will be made to the technique described by Merrifield.
[0211] To make a peptide chain according to the Merrifield procedure, recourse is made to a very porous polymeric resin, on which is immobilized the first C-terminal amino acid of the chain. This amino acid is immobilized on a resin through its carboxyl group and its amine function is protected. The amino acids which are going to form the peptide chain are thus immobilized, one after the other, on the amino group, which is deprotected beforehand each time, of the portion of the peptide chain already formed, and which is attached to the resin. When the whole of the desired peptide chain has been formed, the protective groups of the different amino acids forming the peptide chain are eliminated and the peptide is detached from the resin with the aid of an acid.
[0212] The invention additionally relates to hybrid polypeptides having at least one polypeptide according to the invention, and a sequence of a polypeptide capable of inducing an immune response in man or animals.
[0213] Advantageously, the antigenic determinant is such that it is capable of inducing a humoral and/or cellular response.
[0214] It will be possible for such a determinant to comprise a polypeptide according to the invention in glycosylated form used with a view to obtaining immunogenic compositions capable of inducing the synthesis of antibodies directed against multiple epitopes. Said polypeptides or their glycosylated fragments are likewise part of the invention.
[0215] These hybrid molecules can be formed, in part, of a polypeptide carrier molecule or of fragments thereof according to the invention, associated with a possibly immunogenic part, in particular an epitope of the diphtheria toxin, the tetanus toxin, a surface antigen of the hepatitis $B$ virus (patent $F R$ 79 21811), the VP1 antigen of the poliomyelitis virus or any other viral or bacterial toxin or antigen.
[0216] The procedures for synthesis of hybrid molecules encompass the methods used in genetic engineering for constructing hybrid nucleotide sequences coding for the polypeptide sequences sought. It will be possible, for example, to refer advantageously to the technique for obtainment of genes coding for fusion proteins described by Minton in 1984.
[0217] Said hybrid nucleotide sequences coding for a hybrid polypeptide as well as the hybrid polypeptides according to the invention characterized in that they are recombinant polypeptides obtained by the expression of said hybrid nucleotide sequences are likewise part of the invention.
[0218] The invention likewise comprises the vectors characterized in that they contain one of said hybrid nucleotide sequences. The host cells transformed by said vectors, the transgenic animals comprising one of said transformed cells as well as the procedures for preparation of recombinant polypeptides using said vectors, said transformed cells and/or said transgenic animals are, of course, likewise part of the invention.
[0219] The polypeptides according to the invention, the antibodies according to the invention described below and the nucleotide sequences according to the invention can advantageously be employed in procedures for the detection and/or identification of PWD circovirus, or of porcine circovirus
other than a PWD circovirus, in a biological sample (biological tissue or fluid) capable of containing them. These procedures, according to the specificity of the polypeptides, the antibodies and the nucleotide sequences according to the invention which will be used, will in particular be able to detect and/or to identify a PWD circovirus or a porcine circovirus other than a PWD circovirus or other than the PWD circovirus of type $B$.
[0220] The polypeptides according to the invention can advantageously be employed in a procedure for the detection and/or the identification of PWD circovirus of type A, of type B, of type A or B, or porcine circovirus other than the PWD circovirus of type $B$, or of porcine circovirus other than the PWD circovirus of type A or B , in a biological sample (biological tissue or fluid) capable of containing them, characterized in that it comprises the following steps:
[0221] a) contacting of this biological sample with a polypeptide or one of its fragments according to the invention (under conditions allowing an immunological reaction between said polypeptide and the antibodies possibly present in the biological sample);
[0222] b) demonstration of the antigen-antibody complexes possibly formed.
[0223] In the present description, PWD circovirus, except if a particular mention is indicated, will be understood as designating a PWD circovirus of type A or of type B, and porcine circovirus other than PWD, except if a particular mention is indicated, will be understood as designating a porcine circovirus other than a PWD circovirus of type $A$ and B.
[0224] Preferably, the biological sample is formed by a fluid, for example a pig serum, whole blood or biopsies.
[0225] Any conventional procedure can be employed for carrying out such a detection of the antigen-antibody complexes possibly formed.
[0226] By way of example, a preferred method brings into play immunoenzymatic processes according to the ELISA technique, by immunofluorescence, or radioimmunological processes (RIA) or their equivalent.
[0227] Thus, the invention likewise relates to the polypeptides according to the invention, labeled with the aid of an adequate label such as of the enzymatic, fluorescent or radioactive type.
[0228] Such methods comprise, for example, the following steps:
[0229] deposition of determined quantities of a polypeptide composition according to the invention in the wells of a microtiter plate,
[0230] introduction into said wells of increasing dilutions of serum, or of a biological sample other than that defined previously, having to be analyzed,
[0231] incubation of the microplate,
[0232] introduction into the wells of the microtiter plate of labeled antibodies directed against pig immunoglobulins, the labeling of these antibodies having been carried out with the aid of an enzyme selected from those which are capable of hydrolyzing a substrate by modifying the absorption of the radiation of the latter, at least at a determined wavelength, for example at 550 nm ,
[0233] detection, by comparison with a control test, of the quantity of hydrolyzed substrate.
[0234] The invention likewise relates to a kit or set for the detection and/or identification of PWD circovirus, of porcine circovirus other than a PWD circovirus or of porcine circovi-
rus other than the PWD circovirus of type B, characterized in that it comprises the following elements:
[0235] a polypeptide according to the invention,
[0236] if need be, the reagents for the formation of the medium favorable to the immunological or specific reaction,
[0237] if need be, the reagents allowing the detection of the antigen-antibody complexes produced by the immunological reaction between the polypeptide(s) of the invention and the antibodies possibly present in the biological sample, these reagents likewise being able to carry a label, or to be recognized in their turn by a labeled reagent, more particularly in the case where the polypeptide according to the invention is not labeled,
[0238] if need be, a biological reference sample (negative control) devoid of antibodies recognized by a polypeptide according to the invention,
[0239] if need be, a biological reference sample (positive control) containing a predetermined quantity of antibodies recognized by a polypeptide according to the invention.
[0240] The polypeptides according to the invention allow monoclonal or polyclonal antibodies to be prepared which are characterized in that they specifically recognize the polypeptides according to the invention. It will advantageously be possible to prepare the monoclonal antibodies from hybridomas according to the technique described by Kohler and Milstein in 1975. It will be possible to prepare the polyclonal antibodies, for example, by immunization of an animal, in particular a mouse, with a polypeptide or a DNA, according to the invention, associated with an adjuvant of the immune response, and then purification of the specific antibodies contained in the serum of the immunized animals on an affinity column on which the polypeptide which has served as an antigen has previously been immobilized. The polyclonal antibodies according to the invention can also be prepared by purification, on an affinity column on which a polypeptide according to the invention has previously been immobilized, of the antibodies contained in the serum of pigs infected by a PWD circovirus.
[0241] The invention likewise relates to mono- or polyclonal antibodies or their fragments, or chimeric antibodies, characterized in that they are capable of specifically recognizing a polypeptide according to the invention.
[0242] It will likewise be possible for the antibodies of the invention to be labeled in the same manner as described previously for the nucleic probes of the invention, such as a labeling of enzymatic, fluorescent or radioactive type.
[0243] The invention is additionally directed at a procedure for the detection and/or identification of PWD circovirus, of porcine circovirus other than a PWD circovirus, or other than the PWD circovirus of type B, in a biological sample, characterized in that it comprises the following steps:
[0244] a) contacting of the biological sample (biological tissue or fluid) with a mono- or polyclonal antibody according to the invention (under conditions allowing an immunological reaction between said antibodies and the polypeptides of PWD circovirus, of porcine circovirus other than a PWD circovirus, of porcine circovirus other than the PWD circovirus of type B, possibly present in the biological sample);
[0245] b) demonstration of the antigen-antibody complex possibly formed.
[0246] Likewise within the scope of the invention is a kit or set for the detection and/or the identification of PWD circovirus, of porcine circovirus other than a PWD circovirus or of porcine circovirus other than the PWD circovirus of type $B$, characterized in that it comprises the following components:
[0247] a polyclonal or monoclonal antibody according to the invention, if need be labeled;
[0248] if need be, a reagent for the formation of the medium favorable to the carrying out of the immunological reaction;
[0249] if need be, a reagent allowing the detection of the antigen-antibody complexes produced by the immunological reaction, this reagent likewise being able to carry a label, or being capable of being recognized in its turn by a labeled reagent, more particularly in the case where said monoclonal or polyclonal antibody is not labeled;
[0250] if need be, reagents for carrying out the lysis of cells of the sample tested.
[0251] The present invention likewise relates to a procedure for the detection and/or the identification of PWD, of porcine circovirus other than a PWD circovirus or of porcine circovirus other than the PWD circovirus of type $B$, in a biological sample, characterized in that it employs a nucleotide sequence according to the invention.
[0252] More particularly, the invention relates to a procedure for the detection and/or the identification of PWD circovirus, of porcine circovirus other than a PWD circovirus or of porcine circovirus other than the PWD circovirus of type B , in a biological sample, characterized in that it contains the following steps:
[0253] a) if need be, isolation of the DNA from the biological sample to be analyzed;
[0254] b) specific amplification of the DNA of the sample with the aid of at least one primer, or a pair of primers, according to the invention;
[0255] c) demonstration of the amplification products.
[0256] These can be detected, for example, by the technique of molecular hybridization utilizing a nucleic probe according to the invention. This probe will advantageously be labeled with a nonradioactive (cold probe) or radioactive element.
[0257] For the purposes of the present invention, "DNA of the biological sample" or "DNA contained in the biological sample" will be understood as meaning either the DNA present in the biological sample considered, or possibly the cDNA obtained after the action of an enzyme of reverse transcriptase type on the RNA present in said biological sample.
[0258] Another aim of the present invention consists in a procedure according to the invention, characterized in that it comprises the following steps:
[0259] a) contacting of a nucleotide probe according to the invention with a biological sample, the DNA contained in the biological sample having, if need be, previously been made accessible to hybridization under conditions allowing the hybridization of the probe with the DNA of the sample;
[0260] b) demonstration of the hybrid formed between the nucleotide probe and the DNA of the biological sample.
[0261] The present invention also relates to a procedure according to the invention, characterized in that it comprises the following steps:
[0262] a) contacting of a nucleotide probe immobilized on a support according to the invention with a biological sample, the DNA of the sample having, if need be, previously been made accessible to hybridization, under conditions allowing the hybridization of the probe with the DNA of the sample;
[0263] b) contacting of the hybrid formed between the nucleotide probe immobilized on a support and the DNA contained in the biological sample, if need be after elimination of the DNA of the biological sample which has not hybridized with the probe, with a nucleotide probe labeled according to the invention;
[0264] c) demonstration of the novel hybrid formed in step b).
[0265] According to an advantageous embodiment of the procedure for detection and/or identification defined previously, this is characterized in that, prior to step a), the DNA of the biological sample is first amplified with the aid of at least one primer according to the invention.
[0266] The invention is additionally directed at a kit or set for the detection and/or the identification of PWD circovirus, of porcine circovirus other than the PWD circovirus or of porcine circovirus other than the PWD circovirus of type B, characterized in that it comprises the following elements:
[0267] a) a nucleotide probe according to the invention;
[0268] b) if need be, the reagents necessary for the carrying out of a hybridization reaction;
[0269] c) if need be, at least one primer according to the invention as well as the reagents necessary for an amplification reaction of the DNA.
[0270] The invention likewise relates to a kit or set for the detection and/or the identification of PWD circovirus, of porcine circovirus other than a PWD circovirus or of porcine circovirus other than the PWD circovirus of type B, characterized in that it comprises the following components:
[0271] a) a nucleotide probe, called a capture probe, according to the invention;
[0272] b) an oligonucleotide probe, called a revealing probe, according to the invention,
[0273] c) if need be, at least one primer according to the invention, as well as the reagents necessary for an amplification reaction of the DNA.
[0274] The invention also relates to a kit or set for the detection and/or identification of PWD circovirus, of porcine circovirus other than a PWD circovirus or of porcine circovirus other than the PWD circovirus of type B, characterized in that it comprises the following elements:
[0275] a) at least one primer according to the invention;
[0276] b) if need be, the reagents necessary for carrying out a DNA amplification reaction;
[0277] c) if need be, a component allowing the sequence of the amplified fragment to be verified, more particularly an oligonucleotide probe according to the invention
[0278] The invention additionally relates to the use of a nucleotide sequence according to the invention, of a polypeptide according to the invention, of an antibody according to the invention, of a cell according to the invention, and/or of an animal transformed according to the invention, for the selection of an organic or inorganic compound capable of modulating, inducing or inhibiting the expression of genes, and/or of modifying the cellular replication of PWD circovirus or capable of inducing or of inhibiting the pathologies linked to an infection by a PWD circovirus.
[0279] The invention likewise comprises a method of selection of compounds capable of binding to a polypeptide or one of its fragments according to the invention, capable of binding to a nucleotide sequence according to the invention, or capable of recognizing an antibody according to the invention, and/or capable of modulating, inducing or inhibiting the expression of genes, and/or of modifying the cellular replication of PWD circovirus or capable of inducing or inhibiting the pathologies linked to an infection by a PWD circovirus, characterized in that it comprises the following steps:
[0280] a) contacting of said compound with said polypeptide, said nucleotide sequence, or with a cell transformed according to the invention and/or administration of said compound to an animal transformed according to the invention;
[0281] b) determination of the capacity of said compound to bind to said polypeptide or said nucleotide sequence, or to modulate, induce or inhibit the expression of genes, or to modulate the growth or the replication of PWD circovirus, or to induce or inhibit in said transformed animal the pathologies linked to an infection by PWD circovirus (designated activity of said compound)
[0282] The compounds capable of being selected can be organic compounds such as polypeptides or carbohydrates or any other organic or inorganic compounds already known, or novel organic compounds elaborated by molecular modeling techniques and obtained by chemical or biochemical synthesis, these techniques being known to the person skilled in the art.
[0283] It will be possible to use said selected compounds to modulate the cellular replication of PWD circovirus and thus to control infection by this virus, the methods allowing said modulations to be determined being well known to the person skilled in the art.
[0284] This modulation can be carried out, for example, by an agent capable of binding to a protein and thus of inhibiting or of potentiating its biological activity, or capable of binding to an envelope protein of the external surface of said virus and of blocking the penetration of said virus into the host cell or of favoring the action of the immune system of the infected organism directed against said virus. This modulation can likewise be carried out by an agent capable of binding to a nucleotide sequence of a DNA of said virus and of blocking, for example, the expression of a polypeptide whose biological or structural activity is necessary for the replication or for the proliferation of said virus host cells to host cells in the host animal.
[0285] The invention relates to the compounds capable of being selected by a selection method according to the invention.
[0286] The invention likewise relates to a pharmaceutical composition comprising a compound selected from the following compounds:
[0287] a) a nucleotide sequence according to the invention;
[0288] b) a polypeptide according to the invention;
[0289] c) a vector, a viral particle or a cell transformed according to the invention;
[0290] d) an antibody according to the invention;
[0291] e) a compound capable of being selected by a selection method according to the invention;
possibly in combination with a pharmaceutically acceptable vehicle and, if need be, with one or more adjuvants of the appropriate immunity.
[0292] The invention also relates to an immunogenic and/or vaccine composition, characterized in that it comprises a compound selected from the following compounds:
[0293] a) a nucleotide sequence according to the invention;
[0294] b) a polypeptide according to the invention;
[0295] c) a vector or a viral particle according to the invention; and
[0296] d) a cell according to the invention.
[0297] In one embodiment, the vaccine composition according to the invention is characterized in that it comprises a mixture of at least two of said compounds a), b), c) and d) above and in that one of the two said compounds is related to the PWD circovirus of type A and the other is related to the PWD circovirus of type B.
[0298] In another embodiment of the invention, the vaccine composition is characterized in that it comprises at least one compound a), b), c), or d) above which is related to PWD circovirus of type B. In still another embodiment, the vaccine composition is characterized in that it comprises at least one compound a), b), c), or d) above which is related to PWD circovirus of type B ORF'2.
[0299] A compound related to the PWD circovirus of type $A$ or of type $B$ is understood here as respectively designating a compound obtained from the genomic sequence of the PWD circovirus of type A or of type B.
[0300] The invention is additionally aimed at an immunogenic and/or vaccine composition, characterized in that it comprises at least one of the following compounds:
[0301] a nucleotide sequence SEQ ID No. 23, SEQ ID No. 25, or one of their fragments or homologues;
[0302] a polypeptide of sequence SEQ ID No. 24, SEQ ID No. 26, or one of their fragments, or a modification thereof;
[0303] a vector or a viral particle comprising a nucleotide sequence SEQ ID No. 23, SEQ ID No. 25, or one of their fragments or homologues;
[0304] a transformed cell capable of expressing a polypeptide of sequence SEQ ID No. 24, SEQ ID No. 26 , or one of their fragments, or a modification thereof; or
[0305] a mixture of at least two of said compounds.
[0306] The invention also comprises an immunogenic and/ or vaccine composition according to the invention, characterized in that it comprises said mixture of at least two of said compounds as a combination product for simultaneous, separate or protracted use for the prevention or the treatment of infection by a PWD circovirus, especially of type B.
[0307] In a preferred embodiment, the vaccine composition according to the invention comprises the mixture of the following compounds:
[0308] a pcDNA3 plasmid containing a nucleic acid of sequence $\operatorname{SEQ}$ ID No. 23;
[0309] a pcDNA3 plasmid containing a nucleic acid of sequence SEQ ID No. 25;
[0310] a pcDNA3 plasmid containing a nucleic acid coding for the GM-CSF protein;
[0311] a recombinant baculovirus containing a nucleic acid of sequence SEQ ID No. 23;
[0312] a recombinant baculovirus containing a nucleic acid of sequence SEQ ID No. 25 ; and
[0313] if need be, an adjuvant of the appropriate immunity, especially the adjuvant AIFTM.
[0314] The invention is likewise directed at a pharmaceutical composition according to the invention, for the prevention or the treatment of an infection by a PWD circovirus.
[0315] The invention is also directed at a pharmaceutical composition according to the invention for the prevention or the treatment of an infection by the PWD circovirus of type B.
[0316] The invention likewise concerns the use of a composition according to the invention, for the preparation of a medicament intended for the prevention or the treatment of infection by a PWD circovirus, preferably by the PWD circovirus of type B.
[0317] Under another aspect, the invention relates to a vector, a viral particle or a cell according to the invention, for the treatment and/or the prevention of a disease by gene therapy.
[0318] Finally, the invention comprises the use of a vector, of a viral particle or of a cell according to the invention for the preparation of a medicament intended for the treatment and/ or the prevention of a disease by gene therapy.
[0319] The polypeptides of the invention entering into the immunogenic or vaccine compositions according to the invention can be selected by techniques known to the person skilled in the art such as, for example, depending on the capacity of said polypeptides to stimulate the T cells, which is translated, for example, by their proliferation or the secretion of interleukins, and which leads to the production of antibodies directed against said polypeptides.
[0320] In pigs, as in mice, in which a weight dose of the vaccine composition comparable to the dose used in man is administered, the antibody reaction is tested by taking of the serum followed by a study of the formation of a complex between the antibodies present in the serum and the antigen of the vaccine composition, according to the usual techniques.
[0321] The pharmaceutical compositions according to the invention will contain an effective quantity of the compounds of the invention, that is to say in sufficient quantity of said compound(s) allowing the desired effect to be obtained, such as, for example, the modulation of the cellular replication of PWD circovirus. The person skilled in the art will know how to determine this quantity, as a function, for example, of the age and of the weight of the individual to be treated, of the state of advancement of the pathology, of the possible secondary effects and by means of a test of evaluation of the effects obtained on a population range, these tests being known in these fields of application.
[0322] According to the invention, said vaccine combinations will preferably be combined with a pharmaceutically acceptable vehicle and, if need be, with one or more adjuvants of the appropriate immunity.
[0323] Today, various types of vaccines are available for protecting animals or man against infectious diseases: attenuated living microorganisms (M. bovis - BCG for tuberculosis), inactivated microorganisms (influenza virus), acellular extracts (Bordetella pertussis for whooping cough), recombined proteins (surface antigen of the hepatitis $B$ virus), polysaccharides (pneumococcal). Vaccines prepared from synthetic peptides or genetically modified microorganisms expressing heterologous antigens are in the course of experimentation. More recently still, recombined plasmid DNAs carrying genes coding for protective antigens have been proposed as an alternative vaccine strategy. This type of vaccination is carried out with a particular plasmid originating from a plasmid of $E$. coli which does not replicate in vivo and
which codes uniquely for the vaccinating protein. Animals have been immunized by simply injecting the naked plasmid DNA into the muscle. This technique leads to the expression of the vaccine protein in situ and to an immune response of cellular type (CTL) and of humoral type (antibody). This double induction of the immune response is one of the principal advantages of the vaccination technique with naked DNA.
[0324] The vaccine compositions comprising nucleotide sequences or vectors into which are inserted said sequences are especially described in the international application No. WO 90/11092 and likewise in the international application No. WO 95/11307.
[0325] The constitutive nucleotide sequence of the vaccine composition according to the invention can be injected into the host after having been coupled to compounds which favor the penetration of this polynucleotide into the interior of the cell or its transport to the cell nucleus. The resultant conjugates can be encapsulated in polymeric microparticles, as described in the international application No. WO 94/27238 (Medisorb Technologies International).
[0326] According to another embodiment of the vaccine composition according to the invention, the nucleotide sequence, preferably a DNA, is complexed with DEAE-dextran (Pagano et al., 1967) or with nuclear proteins (Kaneda et al., 1989), with lipids (Felgner et al., 1987) or encapsulated in liposomes (Fraley et al., 1980) or else introduced in the form of a gel facilitating its transfection into the cells (Midoux et al., 1993, Pastore et al., 1994). The polynucleotide or the vector according to the invention can also be in suspension in a buffer solution or be combined with liposomes.
[0327] Advantageously, such a vaccine will be prepared according to the technique described by Tacson et al. or Huygen et al. in 1996 or alternatively according to the technique described by Davis et al. in the international application No. WO 95/11307.
[0328] Such a vaccine can likewise be prepared in the form of a composition containing a vector according to the invention, placed under the control of regulation elements allowing its expression in man or animal. It will be possible, for example, to use, by way of in vivo expression vector of the polypeptide antigen of interest, the plasmid pcDNA3 or the plasmid pcDNA1/neo, both marketed by Invitrogen (R\&D Systems, Abingdon, United Kingdom). It is also possible to use the plasmid V1Jns.tPA, described by Shiver et al. in 1995. Such a vaccine will advantageously comprise, apart from the recombinant vector, a saline solution, for example a sodium chloride solution.
[0329] Pharmaceutically acceptable vehicle is understood as designating a compound or a combination of compounds entering into a pharmaceutical composition or vaccine which does not provoke secondary reactions and which allows, for example, the facilitation of the administration of the active compound, an increase in its duration of life and/or its efflcacy in the body, an increase in its solubility in solution or alternatively an improvement in its conservation. These pharmaceutically acceptable vehicles are well known and will be adapted by the person skilled in the art as a function of the nature and of the mode of administration of the chosen active compound.
[0330] As far as the vaccine formulations are concerned, these can comprise adjuvants of the appropriate immunity which are known to the person skilled in the art, such as, for example, aluminum hydroxide, a representative of the family
of muramyl peptides such as one of the peptide derivatives of N -acetyl muramyl, a bacterial lysate, or alternatively Freund's incomplete adjuvant
[0331] These compounds can be administered by the systemic route, in particular by the intravenous route, by the intramuscular, intradermal or subcutaneous route, or by the oral route. In a more preferred manner, the vaccine composition comprising polypeptides according to the invention will be administered by the intramuscular route, through the food or by nebulization several times, staggered over time.
[0332] Their administration modes, dosages and optimum pharmaceutical forms can be determined according to the criteria generally taken into account in the establishment of a treatment adapted to an animal such as, for example, the age or the weight, the seriousness of its general condition, the tolerance to the treatment and the secondary effects noted. Preferably, the vaccine of the present invention is administered in an amount that is protective against piglet weight loss disease.
[0333] For example, in the case of a vaccine according to the present invention comprising a polypeptide encoded by a nucleotide sequence of the genome of PCV , or a homologue or fragment thereof, the polypeptide will be administered one time or several times, spread out over time, directly or by means of a transformed cell capable of expressing the polypeptide, in an amount of about 0.1 to $10 \mu \mathrm{~g}$ per kilogram weight of the animal, preferably about 0.2 to about $5 \mu \mathrm{~g} / \mathrm{kg}$, more preferably about 0.5 to about $2 \mu \mathrm{~g} / \mathrm{kg}$ for a dose.
[0334] The present invention likewise relates to the use of nucleotide sequences of PWD circovirus according to the invention for the construction of autoreplicative retroviral vectors and the therapeutic applications of these, especially in the field of human gene therapy in vivo.
[0335] The feasibility of gene therapy applied to man no longer needs to be demonstrated and this relates to numerous therapeutic applications like genetic diseases, infectious diseases and cancers. Numerous documents of the prior art describe the means of employing gene therapy, especially through viral vectors. Generally speaking, the vectors are obtained by deletion of at least some of the viral genes which are replaced by the genes of therapeutic interest. Such vectors can be propagated in a complementation line which supplies in trans the deleted viral functions in order to generate a defective viral vector particle for replication but capable of infecting a host cell. To date, the retroviral vectors are amongst the most widely used and their mode of infection is widely described in the literature accessible to the person skilled in the art.
[0336] The principle of gene therapy is to deliver a functional gene, called a gene of interest, of which the RNA or the corresponding protein will produce the desired biochemical effect in the targeted cells or tissues. On the one hand, the insertion of genes allows the prolonged expression of complex and unstable molecules such as RNAs or proteins which can be extremely difficult or even impossible to obtain or to administer directly. On the other hand, the controlled insertion of the desired gene into the interior of targeted specific cells allows the expression product to be regulated in defined tissues. For this, it is necessary to be able to insert the desired therapeutic gene into the interior of chosen cells and thus to have available a method of insertion capable of specifically targeting the cells or the tissues chosen.
[0337] Among the methods of insertion of genes, such as, for example, microinjection, especially the injection of naked plasmid DNA (Derse, D. et al., 1995, and Zhao, T. M. et al., 1996), electroporation, homologous recombination, the use of viral particles, such as retroviruses, is widespread. However, applied in vivo, the gene transfer systems of recombinant retroviral type at the same time have a weak infectious power (insufficient concentration of viral particles) and a lack of specificity with regard to chosen target cells.
[0338] The production of cell-specific viral vectors, having a tissue-specific tropism, and whose gene of interest can be translated adequately by the target cells, is realizable, for example, by fusing a specific ligand of the target host cells to the N -terminal part of a surface protein of the envelope of PWD circovirus. It is possible to mention, for example, the construction of retroviral particles having the CD4 molecule on the surface of the envelope so as to target the human cells infected by the HW virus (YOUNG, J. A. T. et al., Sciences 1990, 250, 1421-1423), viral particles having a peptide hormone fused to an envelope protein to specifically infect the cells expressing the corresponding receptor (KASAHARA, N. et al., Sciences 1994, 266, 1373-1376) or else alternatively viral particles having a fused polypeptide capable of immobilizing on the receptor of the epidermal growth factor (EGF) (COSSET, F. L. et al., J. of Virology 1995, 69, 10, 63146322). In another approach, single-chain fragments of antibodies directed against surface antigens of the target cells are inserted by fusion with the N-terminal part of the envelope protein (VALSESIA-WITTMAN, S. et al., J. of Virology 1996, 70, 3, 2059-2064; TEARINA CHU, T. H. et al., J. of Virology 1997, 71, 1, 720-725).
[0339] For the purposes of the present invention, a gene of interest in use in the invention can be obtained from a eukaryotic or prokaryotic organism or from a virus by any conventional technique. It is, preferably, capable of producing an expression product having a therapeutic effect and it can be a product homologous to the cell host or, alternatively, heterologous. In the scope of the present invention, a gene of interest can code for an (i) intracellular or (ii) membrane product present on the surface of the host cell or (iii) secreted outside the host cell. It can therefore comprise appropriate additional elements such as, for example, a sequence coding for a secretion signal. These signals are known to the person skilled in the art.
[0340] In accordance with the aims pursued by the present invention, a gene of interest can code for a protein corresponding to all or part of a native protein as found in nature. It can likewise be a chimeric protein, for example arising from the fusion of polypeptides of various origins or from a mutant having improved and/or modified biological properties. Such a mutant can be obtained, by conventional biological techniques, by substitution, deletion and/or addition of one or more amino acid residues.
[0341] It is very particularly preferred to employ a gene of therapeutic interest coding for an expression product capable of inhibiting or retarding the establishment and/or the development of a genetic or acquired disease. A vector according to the invention is in particular intended for the prevention or for the treatment of cystic fibrosis, of hemophilia A or B, of Duchenne's or Becker's myopathy, of cancer, of AIDS and of
other bacteria or infectious diseases due to a pathogenic organism: virus, bacteria, parasite or prion. The genes of interest utilizable in the present invention are those which code, for example, for the following proteins:
[0342] a cytokine and especially an interleukin, an interferon, a tissue necrosis factor and a growth factor and especially a hematopoietic growth factor (G-CSF, GMCSF),
[0343] a factor or cofactor involved in clotting and especially factor VIII, von Willebrand's factor, antithrombin III, protein C , thrombin and hirudin,
[0344] an enzyme or an enzyme inhibitor such as the inhibitors of viral proteases,
[0345] an expression product of a suicide gene such as thymidine kinase of the HSV virus (herpesvirus) of type 1,
[0346] an activator or an inhibitor of ion channels,
[0347] a protein of which the absence, the modification or the deregulation of expression is responsible for a genetic disease, such as the CFTR protein, dystrophin or minidystrophin, insulin, ADA (adenosine diaminose), glucocerebrosidase and phenylhydroxylase,
[0348] a protein capable of inhibiting the initiation or the progression of cancers, such as the expression products of tumor suppressor genes, for example the P53 and Rb genes,
[0349] a protein capable of stimulating an immune or an antibody response, and
[0350] a protein capable of inhibiting a viral infection or its development, for example the antigenic epitopes of the virus in question or altered variants of viral proteins capable of entering into competition with the native viral proteins.
[0351] The invention thus relates to the vectors characterized in that they comprise a nucleotide sequence of PWD circovirus according to the invention, and in that they additionally comprise a gene of interest.
[0352] The present invention likewise relates to viral particles generated from said vector according to the invention. It additionally relates to methods for the preparation of viral particles according to the invention, characterized in that they employ a vector according to the invention, including viral pseudoparticles (VLP, virus-like particles).
[0353] The invention likewise relates to animal cells transfected by a vector according to the invention.
[0354] Likewise comprised in the invention are animal cells, especially mammalian, infected by a viral particle according to the invention.
[0355] The present invention likewise relates to a vector, a viral particle or a cell according to the invention, for the treatment and/or the prevention of a genetic disease or of an acquired disease such as cancer or an infectious disease. The invention is likewise directed at a pharmaceutical composition comprising, by way of therapeutic or prophylactic agent, a vector or a cell according to the invention, in combination with a vehicle acceptable from a pharmaceutical point of view.
[0356] Other characteristics and advantages of the invention appear in the examples and the figures.
[0357] The invention is described in more detail in the following illustrative examples. Although the examples may
represent only selected embodiments of the invention, it should be understood that the following examples are illustrative and not limiting.

## Examples

Example 1
Cloning, Sequencing and Characterization of the PWD Circovirus of Type A (PCVA)
[0358] 1. Experimental Procedures
[0359] Experimental reproduction of the infection and its syndrome are provided (cf. FIG. 1).
[0360] A first test was carried out with pigs from a very well-kept farm, but affected by piglet weight loss disease (PWD), likewise called fatal piglet wasting (FPW). Tests carried out with SPF (specific pathogen-free) pigs showed a transfer of contaminant(s) finding expression in a complex pathology combining hyperthermia, retardation of growth, diarrhea and conjunctivitis. The PDRS (porcine dysgenic and respiratory syndrome) virus, an infectious disease due to an arteriovirus) was rapidly isolated from breeding pigs and contact pigs. It should have been possible to attribute all the clinical signs to the presence of the PDRS virus. However, two farm pigs presented signs of FPW without the PDRS virus being isolated. The histological analyses and blood formulas, however, showed that these pigs were suffering from an infectious process of viral origin.
[0361] In a second test, 8 -week SPF pigs were inoculated by the intratracheal route with organ homogenates of two farm pigs suffering from FPW. The inoculated pigs exhibited hyperthermia 8 to 9 days post-infection, then their growth was retarded. Other SPF pigs, placed in contact, had similar, attenuated signs 30 days after the initial experiment. No seroconversion with respect to a European or Canadian strain of PDRS virus was recorded in these animals.
[0362] A third test allowed the syndrome to be reproduced from samples taken from the pigs of the second test.
[0363] Conclusion
[0364] The syndrome is reproduced under the experimental conditions. It is determined by at least one infectious agent, which is transmittable by direct contact. The clinical constants are a sometimes high hyperthermia (greater than or equal to $41.5^{\circ} \mathrm{C}$.) which develops 8 to 10 days after infection. Retardation of the growth can be observed. The other signs are a reversal of the blood formula (reversal of the lymphocyte/polynuclear ratio from $70 / 30$ to $30 / 70$ ) and frequent lesions on the ganglia, especially those draining the respiratory apparatus (ganglionic hypertrophy, loss of structure with necrosis and infiltration by mononucleated or plurinucleated giant cells).

## 2. Laboratory Studies

[0365] Various cell supports including primary pig kidney cells or cell lines, pig testicle cells, monkey kidney cells, pig lymphocytes, pig alveolar macrophages and circulating blood monocytes were used to demonstrate the possible presence of a virus. No cytopathic effect was demonstrated in these cells. On the other hand, the use of a serum of a pig sick after experimental infection allowed an intracellular antigen to be revealed in the monocytes, the macrophages and approximately $10 \%$ of pig kidney ( PK ) cells infected with organ homogenates. This indirect revealing was carried out kinetically at different culture times. It is evident from this that the
antigen initially appears in the nucleus of the infected cells before spreading into the cytoplasm. The successive passages in cell culture did not allow the signal to be amplified.
[0366] Under electron microscopy on organ homogenates, spherical particles labeled specifically by the serum of sick pigs, infected under the experimental conditions, were visualized. The size of these particles is estimated at 20 nm .
[0367] After two passages of these organ homogenates over pig lymphocytes and then three passages over pig kidney or testicle cells, a cytopathic effect developed and was amplified. An adenovirus was visualized in the electron microscope, which, under the experimental conditions, did not reproduce FPW (only a hyperthermia peak was noted 24 to 48 hours after infection, and then nothing more).
[0368] It has been possible to demonstrate DNA bands in certain samples of pigs infected under the experimental conditions and having exhibited signs of the disease (results not shown). A certain connection exists between the samples giving a positive result in cell culture and those having a DNA band.
[0369] Conclusion
[0370] At least two types of virus were demonstrated in the organ homogenates from pigs suffering from FPW. One is an adenovirus, but by itself alone it does not reproduce the disease. The other type of virus is a circovirus and is associated with FPW. This circovirus, of which two types have been isolated and sequenced, designated below PWD circovirus type A (or PCVA) and PWD circovirus of type B (or PCVB) have mutations with respect to the known sequences of circovirus which are nonpathogenic for the pig.

## 3. Cloning and Sequencing of the DNA of the PWD Circovirus of Type A

[0371] Cloning and sequencing of the DNA of PHD circovirus Type $A$ is accomplished by extraction of the replicative form (RF) DNA, followed by cleavage by the Kpn I enzyme and amplification by a pair of primers flanking the Kpn I restriction site. The two strands of DNA are sequenced at least twice by the Sanger method.
[0372] The nucleic sequence of the strand of (+) polarity of the genome of the PWD circovirus of type A (or PCVA), strain FPW, is represented by the sequence SEQ ID No. 1 in the list of sequences, the nucleic acid sequence of the strand of (-) polarity of the genome of the PWD circovirus of type A (or PCVA) being represented by the nucleic acid sequence $3^{\prime} \rightarrow 5^{\prime}$ of FIG. 3 or by the sequence SEQID No. 5 (represented according to the orientation $5^{\prime} \rightarrow 3^{\prime}$ ) in the list of sequences.
[0373] The amino acid sequences SEQ ID No. 10, SEQ ID No. 12 and SEQ ID No. 14 of the list of sequences respectively represent the sequences of proteins encoded by the nucleic sequences of the 3 open reading frames SEQ ID No. 9 (ORF1), corresponding to the REP protein, SEQ ID No. 11 (ORF2) and SEQ ID No. 13 (ORF3), determined from the sequence SEQ ID No. 1 of the strand of (+) polarity or of the nucleic sequence SEQ ID No. 5 of the strand of (-) polarity of the genome of the PWD circovirus of type A.
4. Comparison of the Nucleotide Sequences and Amino Acids of the PWD Circovirus of Type A (or Associated with PWD) which are Obtained with the Corresponding Sequences of MEEHAN and MANKERTZ Circoviruses of Porcine Cell Lines.
[0374] DNA sequences are analyzed using, DNASIS software.

Sequences of Oligonucleotides Used as Primers or Probes in the Detection and/or Identification Procedures

1. Specific Detection of the PWD Circovirus of Type A:
[0375]

SEQ ID No. 46
primer PCV 5: 5' GTG TGC TCG ACA TTG GTG TG $3^{\prime}$;

SEQ ID No. 47
primer PCV 10: 5' TGG AAT GTT AAC GAG CTG AG 3';
2. Specific Detection of the Circovirus of the Cell Lines: [0376]

SEQ ID No. 46
primer PCF 5: 5' GTG TGC TCG ACA TTG GTG TG $3^{\prime}$;
SEQ ID No. 52
primer MEE 1: 5' TGG AAT GTT AAC TAC CTC AA $3^{\prime}$;

## 3. Differential Detection:

[0377] the pairs of primers used are those described, for example, in the paragraphs 1 and 2 above;
4. Detection of the Monomeric Circular Replicative Forms RF:
[0378]

> SEQ ID No. 46 primer PCV 5: 5' GTG TGC TCG ACA TTG GTG TG 3';
> SEQ ID No. 48
> primer PCV 6: 5' CTC GCA GCC ATC TTG GAA TG 3';
5. Detection of the Vectors Carrying the Dimers in Tandem: [0379]
Nar dimer:
primer KS 620: 5' CGC GCG TAA TAC GAC TCA CT ${ }^{\prime}$ '; 49

SEQ ID No. 46
primer PCV 5: 5' GTG TGC TCG ACA TTG GTG TG $3^{\prime}$;

Kpn dimer:
SEQ ID No. 49
primer KS 620: 5' CGC GCG TAA TAC GAC TCA CT 3';
SEQ ID No. 48
primer PCV 6: $5^{\prime} \mathrm{CTC}$ GCA GCC ATC TTG GAA TG $3^{\prime \prime}$;

## 6. Differential Detection:

[0380] The pairs of primers used are those described, for example, in paragraphs 4 and 5 above.
[0381] The procedures using the pairs or primers described in paragraphs 4 and 5 are of particular interest for differentially detecting the circular monomeric forms of specific replicative forms of the virion or of the DNA in replication and the dimeric forms found in the so-called in-tandem molecular constructs.
[0382] The in-tandem constructs of the viral genome (dimers) such as the constructs used for the preparation of the
pBS KS+tandem PCV Kpn I vector, deposited at the CNCM under the number I-1891, 3 Jul. 1997 ( $E$. coli transformed by said vector) are very interesting for their use in methods of production of sufficient quantity of an inoculum formed of DNA, intended for the virus production, this in the absence of a satisfactory virus production protocol in a cell system. These said methods of production using in-tandem constructs of the viral genome will allow the virulence factors to be studied by mutation and by way of consequence will be able to be used for the production of a collection of viruses carrying the mutations indicated in the construction of vectors which will have the appropriate tropism and virulence. These vectors with autoreplicative structure have the sought gene transfer properties, especially for their applications in gene therapy, and in vaccinology.
[0383] Western-Blot Analysis of Recombinant Proteins of the PWD Circovirus of Type A
[0384] The results were obtained using a specific antiserum of the PWD circovirus produced during test 1 (cf. FIG. 1).
[0385] Type of Products Analyzed
[0386] The analyses were carried out on cell extracts of Sf9 cells obtained after infection by the recombinant baculovirus PCV ORF 1.
[0387] The culture of Sf 9 cells was carried out in a $25 \mathrm{~cm}^{2}$ Petri dish according to the standard culture methods for these cells. After centrifugation, the cell pellets are taken up with $300 \mu \mathrm{l}$ of PBS buffer (phosphate saline buffer).
[0388] Electrophoresis (PAGE-SDS)
[0389] The electrophoresis is carried out on the cell extracts of Sf9 cells obtained previously on 5 samples (cf. Table 1 below) under the following conditions:
[0390] \% polyacrylamide gel: $8 \%$; conditions: denaturing
[0391] Voltage: 80 V ; duration: 135 mn .
TABLE 1
Nature of the samples subjected to electrophoresis

|  | Well No. |  |  |  |  |
| :--- | :---: | :---: | :---: | :---: | :---: |
|  | 1 | 2 | 3 | 4 | 5 |
| Sample applied | PM | Raoul | Raoul | Raoul | Raoul |
|  | Rainbow | 24 h | 48 h | 72 h | 96 h |
| $\mu \mathrm{l}$ of sample | 10 | 15 | 15 | 15 | 15 |
| $\mu \mathrm{l}$ of Laemmli | 0 | 5 | 5 | 5 | 5 |
| 4 X |  |  |  |  |  |

Legends to Table 1:
Laemmli 4X: loading buffer
PM Rainbow: molecular-weight markers ( $35,52,77,107,160$ and 250 kD )
Raoul $24 \mathrm{~h}, 48 \mathrm{~h}, 72 \mathrm{~h}$ and 96 h : expression products of the ORF1 of the PWD circovirus of type $A$.
[0392] Western Blot
[0393] After electrophoresis, the bands obtained in the different wells are transferred to nitrocellulose membrane for 1 h at 100 v in a TGM buffer (tris-glycine-methanol).
[0394] The Western blot is carried out under the following conditions:
[0395] 1) Saturation with a solution containing $5 \%$ of skimmed milk; $0.05 \%$ of Tween 20 in a TBS $1 \times$ buffer (tris buffer saline) for 30 min .
[0396] 2) 1st antibody:
[0397] 10 ml of PWD anticircovirus antibody of type A are added diluted to $1 / 100$, then the reaction mixture is incubated for one night at $4^{\circ} \mathrm{C}$. Three washes of 10 $\min$ in TBS $1 \times$ are carried out.
[0398] 3) 2nd antibody:
[0399] 10 ml of pig rabbit P164 antibody anti-immunoglobulins, coupled to peroxidase (Dakopath), are added diluted to $1 / 100$, then the reaction medium is incubated for 3 hours at $37^{\circ} \mathrm{C}$. Three washes of 10 $\min$ in TBS $1 \times$ are carried out.
[0400] 4) Visualization
[0401] The substrate 4-chloro-1-naphthol in the presence of oxygenated water is used for visualization. Results
[0402] The results are shown in FIG. 7.
Kinetics of Appearance of Antibodies Specific for the REP Recombinant Protein of the PWD Circovirus of Type A Expressed in Baculovirus After Infection of Pigs by the PWD Circovirus of Type A (test 4, cf. FIG. 1)
[0403] After infection of the pigs, a sample of serum of each of the infected pigs is taken at different periods expressed in the table by the date of taking (carried out here in the same year) and is then analyzed by Western blot.
[0404] The visualization of the specific antibodies is carried out in the manner described previously.
[0405] The results obtained are shown by Table 2 below.
TABLE 2

| Sample | Kinetics of appearance of specific antibodies |  |  |  |  |  |  | 21/07 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | Pigs | 10/6 | 16/06 | 23/06 | 01/07 | 08/07 | 15/07 |  |
| A3 | 1 |  |  |  |  |  | Neg. |  |
| Control | 2 |  |  |  |  |  | Neg. |  |
| B2 Infec. | 1 | Neg. | Neg. | Neg. | + | + | ++ | +++ |
| RP+ | 2 | Neg. | Neg. | Neg. | Neg. | Neg. | Neg. | Neg. |
|  | 3 | Neg. | Neg. | Neg. | Neg. | + |  | + |
|  | 4 | Neg. | Neg. | Neg. | Neg. | Neg. | Neg. | ++ |

Legends to Table 2:
A3 control: uninfected control animals;
B2 Infec. RP+: animals infected with pig kidney (PK) cells containing the circovirus;
Neg.: negative;
,,++++++ intensity scale of the positive reaction;
$10 / 06,16 / 06,23 / 06,01 / 07,08 / 07,15 / 07,21 / 07$ : dates expressed in day/month on which the different withdrawals of serum were carried out.

## Example 2

Cloning, Sequencing and Characterization of the Type B PWD Circovirus (PCVB)
[0406] The techniques used for cloning, sequencing and characterization of the type B PWD circovirus (PCVB) are those used in Example 1 above for the type A PWD circovirus (PCVA).
[0407] The nucleic acid sequence of the strand of (+) polarity of the genome of the PWD circovirus of type B (or PCVB) is represented by the sequence SEQ ID No. 15 in the sequence listing, the nucleic acid sequence of the strand of $(-)$ polarity of the genome of the PWD circovirus of type B (or PCVB) being represented by the nucleic acid sequence $3^{\prime} \rightarrow 5^{\prime}$ of FIG. 8 or by the sequence SEQ ID No. 19 (represented according to the orientation $5^{\prime} \rightarrow 3^{\prime}$ ) in the sequence listing.
[0408] The amino acid sequences, SEQ ID No. 24, SEQ ID No. 26 and SEQ ID No. 28 of the sequence listing, respectively, represent the sequences of the proteins encoded by the nucleic sequences of the 3 open reading frames SEQ ID No. 23 (ORF'1), corresponding to the REP protein, SEQ ID No. 25 (ORF'2) and SEQ ID No. 27 (ORF'3), determined from the sequence SEQ ID No. 15 of the strand of (+) polarity or from
the nucleic sequence SEQ ID No. 19 of the strand of (-) polarity of the genome of the PWD circovirus of type $B$.

## Example 3

Comparative Analysis of Nucleotide Sequences (ORF1, ORF2 and Genomic) and Amino Acid Sequences Encoded by the ORF1 and the ORF2 of the PWD Circoviruses of Type A (PCVA) and of Type B (PCVB)
[0409] The results expressed in \% of homology are shown in Tables 3 and 4 below.

TABLE 3

| Compared analysis of the amino acid sequences |  |  |
| :---: | :---: | :---: |
| \% homology | ORF1 | ORF2 |
| PCVA/PCVB | 80.4 | 56.2 |

TABLE 4

|  | Compared analysis of the nucleotide sequences |  |  |  |
| :--- | :---: | :---: | :---: | :---: |
|  | Genomic | ORF1 | ORF2 | The remainder |
| \% homology | 70.4 | 80.4 | 60.1 | 66.1 |
| PCVA/PCVB |  |  |  |  |

## Example 4

## Observation of the Disease and Reproduction of the Disease Under Experimental Conditions

[0410] a) Test No. 1: Observation of the Disease
[0411] The objective is to take breeding animals at the start of disease and to place them under experimental conditions to follow the progression of the pathology and describe all the clinical signs thereof. This first test was carried out on 3 breeding pigs aged 10 weeks of which 2 were already ill (suffering from wasting), and on 3 other pigs aged 13 weeks, not having signs of disease. The clinical observation was spread over a period of 37 days. Two pigs of 10 weeks wasted rapidly (pigs 1 and 2, FIG. 9 ) and had to be painlessly killed 5 and 6 days after their arrival. A single pig exhibited hyperthermia over 5 days and diarrhea. Two other pigs exhibited dyspnea and cough, of which one additionally had hyperthermia, greater than $41^{\circ} \mathrm{C}$., for the two first days of its stay. Another pig had retarded growth in the second week (pig 6 , FIG. 9), without any other clinical sign being recorded. On the lesional level, 5 pigs out of 6 exhibited macroscopic lesions of gray pneumonia, the sixth exhibited cicatricial lesions on the lung.
[0412] b) Test No. 2: Reproduction of the Disease from Inocula Prepared in Farm Pigs.
[0413] The two sick pigs in test 1 served to prepare inocula which were tested in test 2 on specific-pathogen-free (SPF) pigs. The SPF pigs were aged 9 weeks at the time of inoculation. The clinical and lesional results are shown in Table 5.

TABLE 5

| Summary of the measurements carried out during experimental reproduction of PWD. (The values of the control animals are reported in brackets, the underlined values indicate a difference between infected animals and control animals) |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | Test Measurement |  |  |  |  |  |
|  | 2 | 3 | 4 | 5 | 6 | 7 |
| Status of the pigs | $\begin{gathered} \text { SPF } \\ \text { CNEVA } \end{gathered}$ | SPF <br> field | $\begin{gathered} \text { SPF } \\ \text { CNEVA } \end{gathered}$ | $\begin{gathered} \text { SPF } \\ \text { CNEVA } \end{gathered}$ | Conventional | Conventional |
| Age | 9 weeks | 6 weeks | 5 weeks | 5 weeks | 5 weeks | 6-7 weeks |
| Number | 4 | 6 | 12 | 8 | 8 | 8 |
| Inoculation route Inoculum titer per pig | Intratracheal route $\mathrm{ND}^{*}$ | Intratracheal route $\mathrm{ND}^{*}$ | Intratracheal + intramuscular route $10^{4.53} \mathrm{TCID}_{50}$ | Intratracheal + intramuscular route $10^{4.53} \mathrm{TCID}_{50}$ | Intratracheal + intramuscular route $10^{4.53} \mathrm{TCID}_{50}$ | Intratracheal + intramuscular route $10^{4.53} \mathrm{TCID}_{50}$ |
| Inockin titer per pig |  |  | per $\mathrm{ml}: 1 \mathrm{ml}$ $\mathrm{IM}+5 \mathrm{ml}$ IT | $\text { per } \mathrm{ml}: 1 \mathrm{ml}$ $\mathrm{IM}+5 \mathrm{ml} \text { IT }$ | $\begin{aligned} & \text { per } \mathrm{ml}: 1 \mathrm{ml} \\ & \mathrm{IM}+5 \mathrm{ml} \text { IT } \end{aligned}$ | $\begin{aligned} & \text { per ml: } 1 \mathrm{ml} \\ & \mathrm{IM}+5 \mathrm{ml} \mathrm{IT} \end{aligned}$ |
| Start of hyperthermia | 10 days | 9-13 days | 12-13 days | 9-14 days | $8-12$ days | 12 days |
|  | post-infection | post-infection | post-infection | post-infection | post-infection | post-infection |
| $\%$ of pigs in hyperthermia** | 100\% | 83\% | 92\% | 100\% | 75\% | 88\% |
| Number of days of hyperthermia per pig** | 7 | 4.5 | 3.3 | 5.8 | 7.5 | 11.6 |
| Maximum <br> temperatures*** <br> Hyperthermia**** <br> \% per week | 40.4 to $41.7^{\circ} \mathrm{C}$. | 40.6 to $42.3^{\circ} \mathrm{C}$. | 40.2 to $41.6^{\circ} \mathrm{C}$. | 40.3 to $40.8^{\circ} \mathrm{C}$. | 40.6 to $42^{\circ} \mathrm{C}$. | 40.2 to $41.9^{\circ} \mathrm{C}$. |
| W1 | 3.5 (3.5) | 17 (36) | 7 (5) | 37 (17) | 16 (17) | 20 (28) |
| W2 | 42 (3.5) | 7 (13) | 13 (1) | $\underline{21(3)}$ | 52 (10) | 37 (28) |
| W3 | 35 (3.5) | 33 (10) | $\underline{28(7)}$ | $62(2)$ | 34 (12) | 79 (17) |
| W4 | $\underline{21 \text { (3.5) }}$ | $\underline{28(7)}$ | 5 (0) | 6 (3) | 25 (22) | 55 (3) |
| DMG: |  |  |  |  |  |  |
| W1 | 928 (1053) | 417 (357) | 564 (620) | 650 (589) | 401 (407) | 509 (512) |
| W2 | 678 (1028) | 428 (617) | 503 (718) | 612 (584) | 294 (514) | 410 (310) |
| W3 | 661 (1000) | 771 (642) | 381 (657) | 520 (851) | 375 (586) | 435 (440) |
| W4 | 786 (1100) | 550 (657) | 764 (778) | 641 (696) | 473 (610) | 451 (681) |

TABLE 5-continued

|  | Summary of the measurements carried out during experimental reproduction of PWD. (The values of the control animals are <br> reported in brackets, the underlined values indicate a difference between infected animals and control animals) |  |  |  |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | 2 | 3 | Test Measurement |

## *ND: not determined

**hyperthermia when the temperature is greater than $40^{\circ} \mathrm{C}$.,
***range of maximum temperatures recorded at the individual level,
****the percentage corresponds to the number of temperature recordings greater than $40^{\circ} \mathrm{C}$. divided by the total number of temperature recordings in the week on all of the pigs.
[0414] In this test, there was no wasting, at the very most a retardation of the growth in the second, third or fourth week after infection. These data illustrate that certain breeding conditions probably favor the expression of the disease.
[0415] c) Tests No. 3 to No. 7: Reproduction of the Experimental Tests
[0416] The increase in the number of the experimental tests on pigs had the mastering and better characterization of the experimental model as an objective. All of the results are presented in Table 5.
[0417] Under the experimental conditions, PWD is thus characterized by a long incubation, of 8 to 14 days, true hyperthermia over 2 to 8 days, a decrease in food consumption and a retardation of the increase in weight on the second, third or fourth week post-infection. The lesional table associated with this clinical expression includes, in the main, ganglionic hypertrophy and lesions of pneumonia.
[0418] Conclusion
[0419] The perfection of this experimental model allows the direct etiological role of the PWD circovirus in the disease to be indisputably demonstrated. In addition, this model is an indispensable tool for the understanding of pathogenic mechanisms and the study of future vaccine candidates.

## Example 5

Demonstration of the Vaccine Composition Protective Efficacy Produced from Nucleic Fragments of

PWD Circovirus Sequence
[0420] 1) Animals Used for the Study
[0421] Piglets having the PWD disease, reproduced under experimental conditions described in paragraph c) of Example 4, were used in a protocol for evaluating the vaccine composition efficacy, comprising nucleic fragments of PWD circovirus sequence
[0422] 2) Tested Vaccine Composition and Vaccination Protocol
[0423] a) Components Used for the Study
[0424] The plasmids were obtained from the pcDNA3 plasmid of INVITROGENE
[0425] pcDNA3ORF-Plasmids
[0426] These plasmids are plasmids which do not carry a PWD circovirus nucleic acid insert and are used as a negative control plasmid.
[0427] pcDNA3ORF1+ Plasmid and pcDNA3ORF2+ Plasmid
[0428] The pcDNA3ORF1 + and pcDNA3ORF2 + plasmids are plasmids which carry a nucleic acid insert of the sequence of the PWD circovirus of TYPE B, and an insert comprising the nucleic acid fragment SEQ ID No. 23 (ORF'1) coding for the Rep protein of sequence SEQ ID No. 24 and an insert comprising the nucleic acid fragment SEQ ID No. 25 (ORF'2) coding for the protein of sequence SEQ ID No. 26, probably corresponding to the capsid protein, respectfully. These nucleic constructs further comprise the ATG initiation codon of the coding sequence of the corresponding protein.
[0429] GMCSF+ Plasmid
[0430] GM-CSF (granulocyte/macrophage colony stimulating factor) is a cytokine which occurs in the development, the maturation and the activation of macrophages, granulocytes and dendritic cells which present an antigen. The beneficial contribution of the GM-CSF in vaccination is considered to be a cellular activation with, especially, the recruitment and the differentiation of cells which present an antigen.
[0431] This pcDNA3-GMCSF+ plasmid carries a nucleic acid insert coding for the granulocyte/macrophage colony stimulation factor, the GM-CSF protein.
[0432] The gene coding for this GM-CSF protein was cloned and sequenced by Inumaru et al. (Immunol. Cell Biol., 1995, 73 (5), 474-476). The pcDNA3-GMCSF + plasmid was obtained by Dr. B. Charley of NRA of Jouy-en-Josas (78, France).
[0433] Recombinant Baculoviruses
[0434] The so-called ORF-baculoviruses are viruses not carrying any insert comprising a nucleic acid fragment capable of expressing a PWD circovirus protein.
[0435] The so-called ORF1+ (BAC ORF1+) or ORF2+ (BAC ORF2+) baculoviruses are recombinant baculoviruses carrying an insert comprising a nucleic acid fragment SEQ ID No. 23 (ORF'1) and an insert comprising the nucleic acid fragment SEQ ID No. 25 (ORF'2), respectively.
[0436] Adjuvant
[0437] The adjuvant supplied by the Seppic Company, a subsidiary of AIR LIQUIDE, is the adjuvant corresponding to the reference AIF SEPPIC
[0438] b) Vaccination Protocol
[0439] Weaned piglets aged 3 weeks are divided into four batches $\mathrm{A}, \mathrm{B}, \mathrm{C}$ and D each comprising 8 piglets.
[0440] Batches A, B and C, aged 3 weeks, each receive a first injection (injection M1) of 1 ml containing 200 micro-
grams of plasmids (naked DNA) in PBS, $\mathrm{pH}: 7.2$, by the intramuscular route for each of the plasmids mentioned below for each batch, then, at the age of 5 weeks, a second injection (injection M2) comprising these same plasmids. A third injection is carried out simultaneously on the other side of the neck. This third injection comprises 1 ml of a suspension containing $5 \times 10^{6}$ cells infected by recombinant baculoviruses and 1 ml of AIF SEPPIC adjuvant.
[0441] Batch A (F1) (Control Batch):

## First Injection

[0442] pcDNA3ORF1-plasmid, pcDNA3ORF2-plasmid and GMCSF+ plasmid.

Second and Third Injection (Simultaneous)
[0443] pcDNA3ORF1-plasmid, pcDNA3ORF2-plasmid and GMCSF+ plasmid;
[0444] Cells transformed by baculoviruses not containing any nucleic acid insert coding for a PWD circovirus protein;
[0445] AIF SEPPIC adjuvant.
[0446] Batch B (F2) (Control Batch):
First Injection
[0447] pcDNA3ORF1-plasmid, pcDNA3ORF2-plasmid and GMCSF+ plasmid;

Second and Third Injection (Simultaneous)
[0448] pcDNA3ORF1-plasmid, pcDNA3ORF2-plasmid and GMCSF+ plasmid;
[0449] Cells transformed by baculoviruses not containing any nucleic acid insert coding for a PWD circovirus protein;
[0450] AIF SEPPIC adjuvant.
[0451] Batch C (F3):
First Injection
[0452] pcDNA3ORF1+ plasmid, pcDNA3ORF2+ plasmid and GMCSF+ plasmid;

Second and Third Injection (Simultaneous)
[0453] pcDNA3ORF1+ plasmid, pcDNA3ORF2 + plasmid and GMCSF+ plasmid;
[0454] Cells transformed by BAC ORF1+ and BACORF2+ recombinant baculoviruses capable of respectively expressing the Rep protein of sequence SEQ ID No. 24 and the protein of sequence SEQ ID No. 26 of the PWD circovirus of TYPE B. Batch D (F4) (control batch): no injection
[0455] The batches of piglets B, C and D are infected (tested) at the age of 6 weeks although batch A is not subjected to the test
[0456] 3) Observation of the Batches
[0457] counting of coughing/sneezing: 15 minutes/batch/
day;
[0458] consistency of fecal matter: every day;
[0459] regular recordings: weekly taking of blood, weigh-
ing;
[0460] weighing of food refuse: 3 times per week;
[0461] calculation of the daily mean gain in weight (dmg);
[0462] The daily mean gains were calculated for each of the batches over a period of 28 days following testing (cf. FIG.
10), an intermediate calculation of the dmg was likewise
carried out for each of the batches over the first and second periods of 14 days. The results obtained are reported below in Table 6.

TABLE 6

|  | Daily mean gains |  |  |  |
| ---: | :---: | :---: | :---: | :---: |
|  | F1 | F2 | F3 | F4 |
| d0-d14 | 411 g | 450 g | 511 g | 461 g |
| d14-d28 | 623 g | 362 g | 601 g | 443 g |
| d0-d28 | 554 g | 406 g | 556 g | 452 g |

[0463] Measurement of hyperthermia
[0464] The measurement of hyperthermia, of greater than $41^{\circ} \mathrm{C}$. (cf. FIG. 11) and greater than $40.2^{\circ} \mathrm{C}$., was carried out for each of the batches over a total period of 28 days following testing. The results obtained, corresponding to the ratio expressed as a percentage between the number of temperature recordings of greater than $41^{\circ} \mathrm{C}$. (or greater than $40.2^{\circ} \mathrm{C}$.) and the total number of temperature recordings carried out on all of the pigs per one-week period are reported below in Tables 7 and 8, respectively, for the hyperthermia measurements of greater than $41^{\circ} \mathrm{C}$. and greater than $40.2^{\circ} \mathrm{C}$.

TABLE 7

|  | Hyperthermia $>41^{\circ} \mathrm{C}$ |  |  |  |
| :---: | :---: | :---: | :---: | :---: |
|  | F1 | F2 | F3 | F4 |
| W1 | 4.1 | 0 | 0 | 0 |
| W2 | 10.7 | 16. | 0 | 8.9 |
| W3 | 4.7 | 27. | 0 | 45. |
| W4 | 0 | 0 | 0 | 7.5 |

TABLE 8

|  | Hyperthermia $>40.2$ |  |  |  |
| :---: | :---: | :---: | :---: | :---: |
|  | F1 | F2 | F3 | F4 |
| W1 | 29.1 | 10.41 | 29.1 | 20.8 |
| W2 | 28.5 | 39.2 | 10.7 | 37.5 |
| W3 | 14.3 | 68.7 | 25.0 | 81.2 |
| W4 | 3.3 | 17.5 | 20.0 | 55 |

[0465] 4) Conclusion
[0466] The recordings carried out clearly show that the animals which received the three injections of a vaccine composition comprising nucleic acid fragments of PWD circovirus according to the invention and/or capable of expressing recombinant proteins of PWD circovirus, in particular of type B, did not exhibit hyperthermia (cf. FIG. 10). These animals additionally did not experience a decline in their growth, the dmgs being comparable to those of uninfected control animals (cf. FIG. 9). They did not exhibit any particular clinical sign.
[0467] These results demonstrate the efficacious protection of the piglets against infection with a PWD circovirus of the invention, the primary agent responsible for PWD or FPW, provided by a vaccine composition prepared from a nucleic acid fragment of the nucleic sequence of PWD circovirus according to the invention, in particular of type $B$, and/or from recombinant proteins encoded by these nucleic acid fragments.
[0468] These results in particular show that the proteins encoded by the ORF1 and ORF2 of PWD circovirus according to the invention are immunogenic proteins inducing an efficacious protective response for the prevention of infection by a PWD circovirus.

## Example 6

Serological Diagnosis of PWD Circovirus by Immunodetermination Using Recombinant Proteins or Synthetic Peptides of PWD Circovirus
[0469] A. Serological Diagnosis with Recombinant Proteins
[0470] The identification and the sequencing of porcine PWD circovirus allow recombinant proteins of PWD circovirus to be produced by the techniques of genetic recombination well known to the person skilled in the art. Using these techniques, recombinant proteins encoded, in particular, by the ORF' 2 of the PWD circovirus, type B, were expressed by transformed Sf9 insect cells and then isolated.
[0471] These recombinant proteins encoded by the ORF'2 are extracted, after culture of the transformed Sf 9 cells, by thermal cell lysis by means of 3 cycles of freezing/thawing to $-70^{\circ} \mathrm{C} . /+37^{\circ} \mathrm{C}$. Healthy Sf9 cells or nontransformed control Sf9 cells are also lysed.
[0472] Two antigenic fractions originating from nontransformed control Sf9 cells and Sf9 cells expressing the ORF'2 are precipitated at $4^{\circ} \mathrm{C}$. by a $60 \%$ plus or minus $5 \%$ saturated ammonium sulfate solution. Determination of total proteins is carried out with the aid of the Biorad kit. 500 ng of control Sf9 proteins and of semipurified Sf9 proteins expressing the ORF'2, in solution in 0.05 M bicarbonate buffer pH 9.6 , are passively adsorbed at the bottom of 3 different wells of a Nunc Maxisorp microplate by incubation for one night at $+4^{\circ}$ C.
[0473] The reactivity of pig sera with respect to each of these antigenic fractions is evaluated by an indirect ELISA reaction of which the experimental protocol is detailed below:
[0474] Saturation step: $200 \mu 1 /$ well of PBS $1 \times / 3 \%$ semiskimmed milk, 1 h 30 incubation at $37^{\circ} \mathrm{C}$.
[0475] Washing: $200 \mu 1 /$ well of PBS $1 \times /$ Tween 20: $0.05 \%, 3$ rapid washes.
[0476] Serum incubation step: $100 \mu 1 /$ well of serum diluted to $1 / 100$ in PBS $1 \times /$ semi-skimmed milk, $1 \% /$ Tween 20 : $0.05 \%, 1 \mathrm{~h}$ incubation at $37^{\circ} \mathrm{C}$.
[0477] Washing: $200 \mu 1 /$ well of PBS1×/Tween 20: $0.05 \%, 2$ rapid washes followed by 2 washes of 5 min .
[0478] Conjugate incubation step: $50 \mu 1 /$ well of rabbit antipig conjugate diluted to $1 / 1000$ in PBS1 $\times$ /semi-skimmed milk, $1 \%$ /Tween $20: 0.05 \%, 1 \mathrm{~h}$ incubation at $37^{\circ} \mathrm{C}$.
[0479] Washing: $200 \mu 1 /$ well of PBS $1 \times /$ Tween 20: $0.05 \%, 2$ rapid washes followed by 2 washes of 5 min .
[0480] Visualization step: $100 \mu 1 /$ well of OPD substrate/ citrate buffer $/ \mathrm{H}_{2} \mathrm{O}_{2}, 15 \mathrm{~min}$ incubation at $37^{\circ} \mathrm{C}$.
[0481] Termination: $50 \mu \mathrm{l} /$ well of $1 \mathrm{~N}_{2} \mathrm{SO}_{4}$.
[0482] Read optical density in a spectrophotometer at 490
nm.

## Results

[0483] The results obtained are shown below in Table 9.
TABLE 9

|  | Reactivity <br> of Pig Serum <br> not inoculated with <br> Circovirus | Reactivity of Pig Serum <br> inoculated with <br> Circovirus |
| :--- | :---: | :---: |
| Antigens | 0.076 | 0.088 |
| Purified Sf9 control | 0.071 | 1.035 |
| Sf expressing purified ORF'2 |  |  |

[0484] The results are expressed in optical density measured in a spectrophotometer at 490 nm during analysis by ELISA of the reactivity of pig sera which are or are not inoculated with the type B PWD circovirus according to the protocol indicated above.
[0485] B. Serological Diagnosis by Synthetic Peptide
[0486] The epitopic mapping of the proteins encoded, for example, by the nucleic sequences ORF1 and ORF2 of the two types of PWD circovirus (types A and B) additionally allowed immunogenic circoviral epitopes to be identified on the proteins encoded by the nucleic sequences ORF'1 and ORF'2 as well as the specific epitopes of the protein encoded by the nucleic acid sequence ORF'2 of the type B PWD circovirus. Four specific epitopes of the type B PWD circovirus and one epitope common to the two types of PWD circovirus situated on the protein encoded by the nucleic sequence ORF'2 were synthesized in peptide form. The equivalent peptides in the circovirus of type A were likewise synthesized. All peptides were evaluated as diagnostic antigens within the context of carrying out a serological test.

Results
[0487] The results obtained are shown in Table 10, below.

TABLE 10

| Results of the evaluation as a diagnostic antigen of synthetic peptides encoded by the nucleic sequences ORF2 and ORF'2 of PWD circovirus of type A and B. |  |  |  |  |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | Type |  |  |  |  | Infected pig serum reactivity Circovirus B |  |  |  |
|  |  |  | Peptide | $\begin{gathered} \text { PWD } \\ \text { circovirus } \end{gathered}$ | Position | $A A$ sequence | $\begin{aligned} & \text { SPF } \\ & \text { D0/D54 } \end{aligned}$ | $\begin{aligned} & \text { Conventional } \\ & 1 \text { Do/D42 } \end{aligned}$ | Conventional $2 \text { D0/D42 }$ | Epitopic specificity |
| SEQ | ID NO: | 29 | 121 | B | 71-85 | VDMMRFNINDFLPPG | +/-, +++ | +/-, +++ | -, +++ | Circovirus |
| SEQ | ID NO: | 55 | 177 | B | 70-84 | NVNELRFNIGQFLPP | +/-, + | +/-, +/- | +/-, - | B |
| SEQ | ID NO: | 30 | 132 | B | 115-129 | QGDRGVGSSAVILDD | +/-, +/- | ++, ++ | +/-, + | Circovirus |

TABLE 10-continued

$+/-,+,++,+++$. Increasing intensities of the reactivities observed in spot peptides on a nitrocellulose membrane. The porcine sera tested are from animals experimentally infected with the circovirus of type $B$ within the animal houses of the cNEvA. Samples are taken from the animals before inoculation on do and 42 days or 54 days after inoculation, on $d 42$, d54.

## Example 7

Characterization of the Specific Epitopes of the PWD Circovirus of type B
[0488] The proteins encoded by the ORF2 of the porcine circoviruses of type A and B were chosen for this study. For each of the ORF2s (types A and B), 56 peptides of 15 amino acids which overlap every 4 amino acids were synthesized, thus covering the whole of the protein (cf. Table 11 below).

TABLE 11

|  | Sequence of amino acids of the 56 peptides of 15 amino acids synthesized from the nucleic sequence ORF'2 (type B) and ORF2 (type of PWD circovirus with their corresponding spot number (cf. FIG. 12) |  |  |  |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | Type B ORF'2 |  |  |  | Type A ORF2 |  |  |  |  |  |
|  |  |  | Spot No. | Sequence |  |  |  |  | Spot No. | Sequence |
| SEQ | ID | NO: 171 | 104 | MTYPRRRYRRRRHRP | SEQ | ID | NO: | 175 | 160 | MTWPRRRYRRRRTRP |
| SEQ | ID | NO: 172 | 105 | RRRYRRRRHRPRSHL | SEQ | ID | NO: | 176 | 161 | RRRYRRRRTRPRSHL |
| SEQ | ID | NO: 173 | 106 | RRRRHRPRSHLGQIL | SEQ | ID | NO: | 177 | 162 | RRRRTRPRSHLGNIL |
| SEQ | ID | NO: 61 | 107 | HRPRSHLGQILRRRP | SEQ | ID | NO: | 84 | 16.3 | TRPRSHLGNILRRRP |
| SEQ | ID | NO: 62 | 108 | SHLGQILRRRPWLVH | SEQ | ID | NO: | 85 | 164 | SHLGNI LRRRPYLVH |
| SEQ | ID | NO: 63 | 109 | QILRRRPWLVHPRHR | SEQ | ID | NO: | 86 | 165 | NILRRRPYLVHPAFR |
| SEQ | ID | NO: 64 | 110 | RRPWLVHPRHRYRWR | SEQ | ID | NO: | 87 | 166 | RRPYLVHPAFRNRYR |
| SEQ | ID | NO: 65 | 111 | LVHPRHRYRWRRKNG | SEQ | ID | NO: | 88 | 167 | LVHPAFRNRYRWRRK |
| SEQ | ID | NO: 66 | 112 | RHRYRWRRKNGIFNT | SEQ | ID | NO: | 89 | 168 | AFRNRYRWRRKTGIF |
| SEQ | ID | NO: 67 | 113 | RWRRKNGIFNTRLSR | SEQ | ID | NO: | 90 | 169 | RYRWRRKTGI FNSRL |
| SEQ | ID | NO: 68 | 114 | KNGIFNTRLSRTFGY | SEQ | ID | NO: | 91 | 170 | RRKTGI FNSRLSREF |
| SEQ | ID | NO: 69 | 115 | FNTRLSRTFGYTVKR | SEQ | ID | NO: | 92 | 171 | GIFNSRLSREFVLTI |

TABLE 11-continued


TABLE 11-continued

[0489] These peptides were synthesized according to the "spot" method which consists of simultaneous synthesis of a large number of peptides on a cellulose solid support, each site of synthesis of a peptide constituting a spot (Synt:em, NIMES). This method involves orientation of the peptides on the plate, these being fixed covalently by the carboxy-terminal end. A spot represents approximately 50 nmol of peptide. [0490] The reference of the spots and corresponding peptide sequences is given in Table 11.
[0491] These membranes were used for immunoreactivity tests with respect to serum of SPF pigs which were or were not infected experimentally with the type B PWD circoviral strain as well as with respect to sera of infected pigs from conventional farms (conventional farms 1 or 2 ). This study allowed specific immunoreactive peptides of the circovirus of type B corresponding to the spots No. 121, No. 132, No. 133 and No. 152 (respectively of amino acid sequences SEQ ID No. 29, SEQ ID No. 30, SEQ ID No. 31 and SEQ ID No. 32) to be demonstrated. An illustration is shown in FIG. 12 where the membranes are visualized with an infected pig serum coming from a conventional farm. Nonspecific immunoreactive peptides of type [lacuna] were likewise demonstrated, among which we shall keep the peptide No. 146 SEQ ID No. 123 which is strongly immunogenic.
[0492] A comparison between the peptide sequences of circoviruses of type A and B (FIG. 13) indicates a divergence ranging from 20 to $60 \%$ for the specific immunoreactive peptides of the type B, and a weaker divergence (13\%) between the nonspecific peptides.

## Example 8

Protection of Swine From Post-Weaning Multisystemic Wasting Syndrome (PMWS) Conferred by
Procine Circovirus Type B (PCV-B) ORF'2 Protein.
[0493] The ORF' 1 -encoded protein (REP) and ORF'2-encoded putative capsid protein of PCV-B were expressed, either in insect cells by recombinant baculovirus vectors, or in
mammalian cell lines by transfection with plasmidic expression vectors. These two circovirus-derived proteins were detectable in both expression systems. As evaluated by weight gains, hyperthermia and absence of lesions following challenge, the pigs were protected against a virulent circovirus challenge after one first DNA immunization with plasmids directing ORF' 2 protein and GM-CSF expression and a second injection, 15 days later, with the same plasmid preparation plus the ORF' 2 recombinant protein. A lower level of protection was observed when the pigs were vaccinated with ORF'1 protein, as opposed to pigs vaccinated with ORF'2 protein.
A. Development of an Experimental Model of PMWS in Swine:
[0494] Eight 3 week-old SPF pigs were inoculated intratracheally ( 5 ml ) and intramuscularly ( 1 ml ).

## B. Production and Control of PCV-B Plasmids:

[0495] PCV-B ORF'1 and ORF'2 genes, isolated from PCV-B challenge strain, was cloned into vector plasmid pcDNA3.1. All constructs were validated through a partial sequencing of the PCV-B genes in the final plasmids and expression control by immunoperoxidase on PK15 cells respectively transfected with each plasmid, using swine polyclonal antibodies.
[0496] Plasmid encoding GM-CSF has been co-administered.

## C. Construction of Recombinant Baculoviruses:

[0497] ORF'1 and ORF'2 proteins were expressed under polyhedrin promoter control. Recombinant proteins were detected by western-blot using swine polyclonal antibodies.

## D. Vaccination and Challenge:

[0498] Four groups of 7 pigs were vaccinated intramuscularly at day 0 (Do), two weeks later, they received the same plasmid preparation plus the recombinant baculovirus.
E. Monitoring:
[0499] All groups of pigs were housed in isolated experimental units with air filtration and low air pressure. Clinical observations and rectal temperatures were recorded every day. The pigs were weighed weekly.

## F. Conclusions

[0500] Expression of PCV-B ORF' 2 or PCV-B ORF' 1 in swine resulted in a significantly enhanced level of protection as evaluated by weight evolution and body temperature evolution following challenge with PCV-B circovirus. These results are summarized in FIGS. 14 and 15.
[0501] The invention described herein may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The specific embodiments previously described are therefore to be considered as illustrative of, and not limiting, the scope of the invention. Additionally, the disclosure of all publications and patent applications cited above and below, including International Patent Application No. PCT/FR98/02634, filed Dec. 4, 1998, and published as International Publication No. WO 99/29871 on Jun. 17, 1999, are expressly incorporated herein by reference in their entireties to the same extent as if each were incorporated by reference individually.

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| Leu Val Gly Arg Trp Pro Asn Ala Phe Lys Ala Leu Phe Cys Val lys65 70 <br> 75 80 |  |
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| $\begin{array}{rl}\text { Phe Pro Val Ser Trp Cys Phe Leu Ser Tyr Gln Leu Leu Ser Pro Trp } \\ 100 & 105\end{array}$ |  |
| $\begin{gathered} \text { Met Ser Ile Ser His Pro Ala Gly Arg Phe Trp Pro Phe Arg Leu Ser } \\ 115 \\ 120 \end{gathered}$ |  |
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| His Ala Arg Leu Lys Ala Ser Gly Leu Ser Val Gln Phe Gly Leu Leu  <br> 195 200 |  |
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| Trp | Ser | $\begin{aligned} & \text { Leu } \\ & 435 \end{aligned}$ | Leu | Leu | Pro | Gly | $\begin{aligned} & \text { Leu } \\ & 440 \end{aligned}$ | Val | Glu | LYs | Ile | $\begin{aligned} & \text { Leu } \\ & 445 \end{aligned}$ | Pro | Ser | Pro |
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| Ala | Ser | Thr |  | Asp | Ser | Thr | Leu | Met | $\begin{aligned} & \text { Gly } \\ & 490 \end{aligned}$ | Leu | His | Ser | Arg | $\begin{aligned} & \text { Thr } \\ & 495 \end{aligned}$ | Asp |
| Glu | Glu | Pro | $\begin{aligned} & \text { Ser } \\ & 500 \end{aligned}$ | Tyr | Leu | Asn | Glu | $\begin{aligned} & \text { Leu } \\ & 505 \end{aligned}$ | Phe | Ala |  | Ile | $\begin{aligned} & \text { Ser } \\ & 510 \end{aligned}$ | Ser | Val |
| Arg | Arg | $\begin{aligned} & \text { Glu } \\ & 515 \end{aligned}$ | Ala | Gly | Asp | Thr | $\begin{aligned} & \text { Val } \\ & 520 \end{aligned}$ | Thr | Glu | Ser | Pro | $\begin{aligned} & \text { Pro } \\ & 525 \end{aligned}$ | Thr | Tyr | $\operatorname{Trp}$ |
| Ile | $\begin{aligned} & \mathrm{His} \\ & 530 \end{aligned}$ | Asp | Glu | Gly | Ser | $\begin{aligned} & \text { Ser } \\ & 535 \end{aligned}$ | Thr | Glu |  | Ile | $\begin{aligned} & \text { Ala } \\ & 540 \end{aligned}$ | Ala | Pro | Ala | Pro |
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Gly Lys Lys Ile Ile Lys Asn Thr Ala Leu Phe Thr Gln Phe Leu Thr


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70
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Glu Gly His Ile Leu Ile Glu Cys Gly Ala Pro Arg Asn Gln Gly Lys
Arg Ser Asp Leu Ser Thr Ala Val Ser Thr Leu Leu Glu Thr Gly Ser
Leu Val Thr Val Ala Glu Gln Phe Pro Val Thr Tyr Val Arg Asn Phe
130
135

| Arg Gly Leu Ala Glu Leu Leu Lys Val Ser Gly Lys Met Gln Gln Arg |  |  |  |
| :--- | ---: | ---: | ---: |
| 145 | 150 | 155 | 160 |


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210
215


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Leu Phe Pro Tyr Lys Ile Asn Tyr
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Pro Arg Leu Ser Lys Ile Thr Gly Pro Leu Ala Leu Pro Thr Thr Gly
cgg gcc cac tat gac gtg tac agc tgt ctt cca atc acg ctg ctg cat
Arg Ala His Tyr Asp Val Tyr Ser Cys Leu Pro Ile Thr Leu Leu His
ctt ccc gct cac ttt caa aag ttc agc cag ccc geg gaa att tct cac Leu Pro Ala His Phe Gln Lys Phe Ser Gln Pro Ala Glu Ile Ser His $50 \quad 55$ 60
ata cgt tac agg aaa ctg ctc ggc tac agt cac caa aga ccc cgt ctc Ile Arg Tyr Arg Lys Leu Leu Gly Tyr Ser His Gln Arg Pro Arg Leu $65-70 \quad 75 \quad 80$
caa aag ggt act cac agc agt aga cag gtc gct gcg ctt ccc ctg gtt Gln Lys Gly Thr His Ser Ser Arg Gln Val Ala Ala Leu Pro Leu Val 859095
cog cgg agc tcc aca ctc gat aag tat gtg gcc ttc ttt act gca gta Pro Arg Ser Ser Thr Leu Asp Lys Tyr Val Ala Phe Phe Thr Ala Val
Pro Arg Ser Ser Thr Leu Asp Lys Tyr Val Ala Phe Phe Thr Ala Val


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| <220> FEATURE: |  |
| <221> NAME/KEY: | CDS |
| <222> LOCATION: | (271) . . 360 ) |
| <220> FEATURE: |  |
| <221> NAME/KEY: | CDS |
| <222> LOCATION: | (364) . . (417) |
| <220> FEATURE: |  |
| <221> NAME/KEY: | CDS |
| <222> LOCATION: | (421) . . (447) |
| <220> FEATURE: |  |
| <221> NAME/KEY: | CDS |
| <222> LOCATION: | (451) . . (471) |
| <220> FEATURE: |  |
| <221> NAME/KEY: | CDS |
| $<222$ L LOCATION: | (475) . . (510) |
| <220> FEATURE: |  |
| <221> NAME/KEY: | CDS |
| <222> LOCATION: | (514) . . (516) |
| <220> FEATURE: |  |
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| <222> LOCATION: | (520) . . (729) |
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| <221> NAME/KEY: | CDS |
| <222> LOCATION: | (733) . . 753 ) |
| <220> FEATURE: |  |
| <221> NAME/KEY: | CDS |
| <222> LOCATION: | (757) . . (759) |
| $<220>$ FEATURE: |  |
| <221> NAME/KEY: | CDS |
| $<222$ ¢ LOCATION: | (763) . . (804) |
| <220> FEATURE: |  |
| <221> NAME/KEY: | CDS |
| <222> LOCATION: | (808) . . (861) |
| <220> FEATURE: |  |
| <221> NAME/KEY: CDS |  |
| <222> LOCATION: | (865) . . 988 ) |
| <220> FEATURE: |  |
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| $<222>$ LOCATION: | (1237) . . (1359) |
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| <221> NAME/KEY: CDS |  |
| <222> LOCATION: | (1363) . . (1476) |
| <220> FEATURE: |  |
| <221> NAME/KEY: CDS |  |
| <222> LOCATION: (1480) . (1737) |  |
| <220> FEATURE: |  |
| <221> NAME/KEY: CDS |  |
| $<222$ ¢ LOCATION: | (1741) . . 1767 ) |
| <400> SEQUENCE: | 15 |




$<210>$ SEQ ID NO 16
$<211>$ LENGTH: 569
$<212>$ TYPE: PRT
$<213>$ ORGANISM: TYpe B PWD circovirus
$<400>$ SEQUENCE: 16



$<210>$ SEQ ID NO 17
$<211>$ LENGTH: 542
$<212>$ TYPE: PRT
$<213>$ ORGANISM: TYpe B PWD circovirus
$<400>$ SEQUENCE: 17


|  | 210 |  |  |  | 215 |  |  |  |  | 220 |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\begin{aligned} & \text { Ile } \\ & 225 \end{aligned}$ | Asp | Cys | Arg Asp | $\begin{aligned} & \text { Arg } \\ & 230 \end{aligned}$ | Trp | Asn | cys | Thr | Phe 235 | Phe | Gly | Pro | $\mathrm{Gln}$ | $\begin{aligned} & \text { Tyr } \\ & 240 \end{aligned}$ |
| Ser | Asp | Tyr | $\begin{array}{r} G \ln \operatorname{Gln} \\ 245 \end{array}$ | Ser | Asp | ro | Val | $\begin{aligned} & \text { Gly } \\ & 250 \end{aligned}$ | Met | Val | Leu | Leu | $\begin{aligned} & \text { Asn } \\ & 255 \end{aligned}$ | Cys |
| Cys | Pro | Ser | $\begin{aligned} & \text { Cys Arg } \\ & 260 \end{aligned}$ | Ser | Ser | eu | $\begin{aligned} & \text { Ser } \\ & 265 \end{aligned}$ | Glu | Asp | Tyr | Phe | $\begin{aligned} & \text { Leu } \\ & 270 \end{aligned}$ | Gly | Ile |
| Leu | Glu | $\begin{aligned} & \text { Glu } \\ & 275 \end{aligned}$ | cys Tyr | Arg | Thr | $\begin{aligned} & \text { Ile } \\ & 280 \end{aligned}$ | His | Gly | Gly | Arg | $\begin{aligned} & \text { Gly } \\ & 285 \end{aligned}$ | Pro | Val | Arg |
| His | $\begin{aligned} & \text { Pro } \\ & 290 \end{aligned}$ | Phe | Pro Pro | Met | $\begin{aligned} & \text { Pro } \\ & 295 \end{aligned}$ | Asn | Lys | Leu | Leu | $\begin{aligned} & \text { Ser } \\ & 300 \end{aligned}$ | Leu | Phe | Tyr | His |
| $\begin{aligned} & \text { Phe } \\ & 305 \end{aligned}$ | Val | Met | Val Phe | $\begin{aligned} & \text { Ile } \\ & 310 \end{aligned}$ | Ile | His | Gly | Leu | $\begin{aligned} & \text { Ser } \\ & 315 \end{aligned}$ | Gly | Gly | Ser | Leu | $\begin{aligned} & \text { Lys } \\ & 320 \end{aligned}$ |
| Leu | Asn | Ser L | Leu Asn $325$ | Cys | Thr | $\text { Tyr } \mathrm{M}$ | Met | $\begin{aligned} & \mathrm{Val} \\ & 330 \end{aligned}$ | Thr | Arg | Ile | Leu | $\begin{aligned} & \text { Tyr } \\ & 335 \end{aligned}$ | Ser |
| Trp | Ser | Tyr | Ile Leu $340$ | Phe | Ser |  | $\begin{aligned} & \text { Ala } \\ & 345 \end{aligned}$ | Val | Pro | Arg | Pro | $\begin{aligned} & \text { Thr } \\ & 350 \end{aligned}$ | Trp | Ser |
| Thr | Phe | $\begin{aligned} & \text { Pro } \\ & 355 \end{aligned}$ | Ala Val | Cys | Ser | $\begin{aligned} & \text { Leu } \\ & 360 \end{aligned}$ | Ser | His | Ser | Trp | $\begin{aligned} & \text { Phe } \\ & 365 \end{aligned}$ | Leu | Leu | Leu |
| Phe | $\begin{aligned} & \text { Gly } \\ & 370 \end{aligned}$ | $\operatorname{Trp} L$ | Lys Ser | Ile | $\begin{aligned} & \text { Val } \\ & 375 \end{aligned}$ | $\text { Lys } S$ | Ser | Arg | Thr | $\begin{aligned} & \text { Gly } \\ & 380 \end{aligned}$ | Leu | Gly | Val | Lys |
| $\begin{aligned} & \text { Tyr } \\ & 385 \end{aligned}$ | Arg | Glu | Trp Glu | $\begin{aligned} & \text { Lys } \\ & 390 \end{aligned}$ | Gly | $r p$ | Val | Met | $\begin{aligned} & \text { Val } \\ & 395 \end{aligned}$ | Trp | Arg | Glu | Glu | $\begin{aligned} & \mathrm{Val} \\ & 400 \end{aligned}$ |
| Arg | Ala | Val | Ala Phe 405 | Val | Thr | Lys | Leu | $\begin{aligned} & \text { Ser } \\ & 410 \end{aligned}$ | Ser | Lys | Ile | Thr | $\begin{aligned} & \text { Ala } \\ & 415 \end{aligned}$ | Leu |
| Glu | Pro | hr | Pro Leu $420$ | Ser | ro | rp | $\begin{aligned} & \text { Val } \\ & 425 \end{aligned}$ | Ile | Gly | Glu | Gln | $\begin{aligned} & \text { Gly } \\ & 430 \end{aligned}$ | $\mathrm{Gln}$ | Asn |
| Ser | Thr | $\begin{aligned} & \text { Leu } \\ & 435 \end{aligned}$ | Thr Phe | Leu | Ile | $\begin{aligned} & \text { Leu } \\ & 440 \end{aligned}$ | Tyr | Ser | Lys | Gly | $\begin{aligned} & \text { Thr } \\ & 445 \end{aligned}$ | Glu | Arg | Gly |
| Phe | Asp <br> 450 | Pro | Pro Pro | Gly | $\begin{aligned} & \text { Gly } \\ & 455 \end{aligned}$ | Arg | Lys | Ser | Leu | $\begin{aligned} & \text { Ile } \\ & 460 \end{aligned}$ | Leu | Asn | Leu | Ile |
| Met $465$ | Ser | hr | Ala Gln | $\begin{aligned} & \text { Glu } \\ & 470 \end{aligned}$ | Gly | al | Leu | 'hr | $\begin{aligned} & \text { Val } \\ & 475 \end{aligned}$ | Val | Arg | Leu | Thr | $\begin{aligned} & \text { Val } \\ & 480 \end{aligned}$ |
| TYr | Pro | Lys | $\begin{aligned} \text { Val Arg } \\ 485 \end{aligned}$ | Glu | Arg | Arg | Val | $\begin{aligned} & \text { Leu } \\ & 490 \end{aligned}$ | Lys | Met | Pro | Phe | Phe <br> 495 | Leu |
| Leu | Gln | Arg | $\begin{aligned} & \text { Arg Trp } \\ & 500 \end{aligned}$ | Arg | Gly | $\operatorname{Trp}$ | $\begin{aligned} & \text { Thr } \\ & 505 \end{aligned}$ | Ser | Gln | Gly | Arg | $\begin{aligned} & \text { Arg } \\ & 510 \end{aligned}$ | Arg | Arg |
| Ile | Trp | $\begin{aligned} & \text { Pro } \\ & 515 \end{aligned}$ | Arg Trp | Leu | Arg | $\begin{aligned} & \text { Gly } \\ & 520 \end{aligned}$ | Arg | Cys | Leu | Leu | $\begin{aligned} & \text { Leu } \\ & 525 \end{aligned}$ | Arg | Arg | Leu |
| Leu | $\begin{aligned} & \text { Gly } \\ & 530 \end{aligned}$ | Tyr | Val Ile | Ser | $\begin{aligned} & \text { Glu } \\ & 535 \end{aligned}$ | Asn | Glu | Arg | Ser | $\begin{aligned} & \text { Ala } \\ & 540 \end{aligned}$ | Leu | Val |  |  |

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<210> SEQ ID NO 18
<211> LENGTH: 566
<212> TYPE: PRT
<213> ORGANISM: Type B PWD circovirus
<400> SEQUENCE: 18
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$<210>$ SEQ ID NO 19
$<211>$ LENGTH: 1767
$<212>$ TYPE: DNA
$<213>$ ORGANISM: TYpe B PWD circovirus
$<400>$ SEQUENCE: 19
aatacttaca gcgcacttct ttcgttttca gatatgacgt atccaaggag gcgttaccga 60
agaagaagac accgcceccg cagccatctt ggccagatce tcegcegccg cecctggetc 120gtccaccccc gccaccgtta cegctggaga aggaaaaatg gcatcttcaa cacccgcctc 180
tcccgcacet teggatatac tgtcaagcga accacagtca gaacgccctc ctgggcggtg 240
gacatgatga gattcaatat taatgacttt cttcccccag gaggggggtc aaacccccgc 300
tctgtgccct ttgaatacta cagaataaga aaggttaagg ttgaattctg gccctgctcc $\quad 360$
ccgatcaccc agggtgacag gggagtgggc tccagtgctg ttattttaga tgataacttt 420
gtaacaaagg ccacagccet cacctatgac ccctatgtaa actactcctc cogccatacc 480
ataacccagc cottctccta ccactcccgg tactttaccc ccaaacctgt cetagatttc 540
actattgatt acttccaacc aaacaacaaa agaaaccagc tgtggctgag actacaaact 600
gctggaaatg tagaccacgt aggcctcggc actgcgttcg aaaacagtat atacgaccag 660
gaatacaata tccgtgtaac catgtatgta caattcagag aatttaattt taaagacccc 720
ccacttaacc cttaatgaat aataaaacc attacgaagt gataaaaag actcagtaat 780
ttatttcata tggaaattca gggcatgggg gggaaagggt gacgaactgg cccccttcct 840
cogtggattg ttctgtagca ttcttccaaa ataccaagga agtaatcctc cgataaagag 900
cttctacagc tgggacagca gttgaggagt accattccaa cggggtctga ttgctggtaa 960
tcagaatact gegggccaaa aaaggtacag ttccaccttt agtctctaca gtcaatggat 1020
atcgatcaca cagtctcagt agatcatccc agggcagcca gccataaaag tcatcaataa 1080
caaccacttc ttcaccatgg taaccatcce accacttgtt tctaggtggt ttccagtatg 1140
tggtttccgg gtctgcaaaa ttagcagcce atttgctttt accacaccca ggtggcecca 1200
caatgacgtg tacattagtc ttccaatcac gettctgcat tttcccgctc actttcaaaa 1260

| ttcagccag | ccogeggaaa | tttctgacaa | acgttacagg | gtgctgctct | caacggtca | 1320 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| ccagactccc | getctccaac | aaggtactca | cagcagtaga | caggtcactc | cgttgtccct | 1380 |
| gagatctagg | agctccacac | tccatcagta | agttgcettc | tttactgcag | tattctttat | 1440 |
| tctgetgatc | tgttcotttc | getttctcga | tgtggcagcg | ggcacccaaa | taccacttca | 1500 |
| ctttattaaa | agtctgcttc | ttcacaaaat | tagcgaaccc | ctggaggtga | ggtgttcgtc | 1560 |
| cttcctcatt | accctcctcg | ccaacaataa | aataatcaaa | tagggatatt | ggaagatccc | 1620 |
| gtattttctt | gegctcgtct | tcggaaggat | tattcagagt | gaacacccac | cttttatggg | 1680 |
| gttggggtcc | gettcttcca | ttcttcttgc | tgggcatgtt | gctgctgagg | tgctgccgag | 1740 |
| gtgctgccgc | tgecgaagtg | cgctggt |  |  |  | 1767 |

$<210>$ SEQ ID NO 20
$<211>$ LENGTH: 567
$<212>$ TYPE: PRT
$<213>$ ORGANISM: TYpe B PWD circovirus
$<400>$ SEQUENCE $: 20$


$<210>$ SEQ ID NO 21
$<211>$ LENGTH: 566
$<212>$ TYPE: PRT
$<213>$ ORGANISM: TYpe B PWD circovirus
$<400>$ SEQUENCE : 21


- continued


$<210>$ SEQ ID NO 22
$<211>$ LENGTH: 569
$<212>$ TYPE : PRT
$<213>$ ORGANISM: TYpe B PWD circovirus
$<400>$ SEQUENCE: 22



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<210> SEQ ID NO 23
<211> LENGTH: 945
<212> TYPE: DNA
<213> ORGANISM: Type B PWD circovirus
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1) .. (942)
<400> SEQUENCE: 23
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atg ccc agc aag aag aat gga aga agc gga ccc caa ccc cat aaa agg
Met Pro Ser Lys Lys Asn Gly Arg Ser Gly Pro Gln Pro His Lys Arg
1 5 10 15
tgg gtg ttc act ctg aat aat cct tcc gaa gac gag cgc aag aaa ata
Trp Val Phe Thr Leu Asn Asn Pro Ser Glu Asp Glu Arg Lys Lys Ilecgg gat ctt cca ata tcc cta ttt gat tat ttt att gtt ggc gag gag144Arg Asp Leu Pro Ile Ser Leu Phe Asp Tyr Phe Ile Val Gly Glu Glu354045
ggt aat gag gaa gga cga aca cct cac ctc cag ggg ttc get aat ttt192$\begin{array}{cl}\text { Gly Asn Glu Glu Gly Arg Thr Pro } \\ 50 & 55\end{array}$gtg aag aag cag act ttt aat aaa gtg aag tgg tat ttg ggt gcc cgc240Val Lys Lys Gln Thr Phe Asn Lys Val Lys Trp Tyr Leu Gly Ala Arg$65 \quad 70 \quad 75 \quad 10$96
Trp Val Phe Thr Leu Asn Asn Pro Ser Glu Asp Glu Arg Lys Lys Ile
tgc cac atc gag aaa gcg aaa gga aca gat cag cag aat aaa gaa tac ..... 288
Cys His Ile Glu Lys Ala Lys Gly Thr Asp Gln Gln Asn Lys Glu Tyr
tgc agt aaa gaa ggc aac tta ctg atg gag tgt gga gct cet aga tct336
Cys Ser Lys Glu Gly Asn Leu Leu Met Glu Cys Gly Ala Pro Arg Ser100105110
cag gga caa cgg agt gac ctg tct act gct gtg agt acc ttg ttg gag ..... 384Gln Gly Gln Arg Ser Asp Leu Ser Thr Ala Val Ser Thr Leu Leu Glu
115 120 ..... 125
agc ggg agt ctg gtg acc gtt gca gag cag cac cet gta acg ttt gtc ..... 432Ser Gly Ser Leu Val Thr Val Ala Glu Gln His Pro Val Thr Phe Val130135140
aga aat ttc cge ggg ctg gct gaa ctt ttg aaa gtg agc ggg aaa atg ..... 480Arg Asn Phe Arg Gly Leu Ala Glu Leu Leu Lys Val ser Gly Lys Met
145150155160
cag aag cgt gat tgg aag act aat gta cac gtc att gtg ggg cca cct 528Gln Lys Arg Asp Trp Lys Thr Asn Val His Val Ile Val Gly Pro Pro165170175
ggg tgt ggt aaa agc aaa tgg gct gct aat ttt gca gac ccg gaa acc ..... 576Gly Cys Gly Lys Ser Lys Trp Ala Ala Asn Phe Ala Asp Pro Glu Thr180185190
aca tac tgg aaa cca cct aga aac aag tgg tgg gat ggt tac cat ggt528624
Thr Tyr Trp Lys Pro Pro Arg Asn Lys Trp Trp Asp Gly Tyr His Gly
195200205gaa gaa gtg gtt gtt att gat gac ttt tat ggc tgg ctg ccc tgg gat672Glu Glu Val Val Val Ile Asp Asp Phe Tyr Gly Trp Leu Pro Trp Asp$210 \quad 215 \begin{aligned} & 220\end{aligned}$
gat cta ctg aga ctg tgt gat cga tat cca ttg act gta gag act aaa720Asp Leu Leu Arg Leu Cys Asp Arg Tyr Pro Leu Thr Val Glu Thr Lys768

ggt gga act gta cet ttt thg gcc egc agt att ctg att acc agc aat
ggt gga act gta cct ttt ttg gcc cgc agt att ctg att acc agc aat$\begin{array}{rl}\text { Gly Gly Thr Val Pro Phe Leu Ala Arg Ser Ile Leu Ile Thr Ser Asn } \\ 245 & 250\end{array}$
cag acc ccg ttg gaa tgg tac tcc tca act get gtc cca gct gta gaa ..... 816Gln Thr Pro Leu Glu Trp Tyr Ser Ser Thr Ala Val Pro Ala Val Glu
get ctt tat cgg agg att act tcc ttg gta ttt tgg aag aat get aca ..... 864Ala Leu Tyr Arg Arg Ile Thr Ser Leu Val Phe Trp Lys Asn Ala Thr285
gaa caa tcc acg gag gaa ggg gge cag ttc gtc acc ctt tcc ccc cca ..... 912Glu Gln Ser Thr Glu Glu Gly Gly Gln Phe Val Thr Leu ser Pro Pro290295300tge cet gaa ttt cea tat gaa ata aat tac tga945
Cys Pro Glu Phe Pro Tyr Glu Ile Asn Tyr
305 ..... 310


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<210> SEQ ID NO 25
<211> LENGTH: 702
<212> TYPE: DNA
<213> ORGANISM: TYpe B PWD circovirus
<220> FEATURE:
<221> NAME/KEY: CDS
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$<222>$ LOCATION: (1) .. (699)
$<400>$ SEQUENCE: 25
Pro Pro Gly Gly Gly Ser Asn Pro Arg Ser Val Pro Phe Glu Tyr Tyr
aga ata aga aag gtt aag gtt gaa ttc tgq ccc tgc tcc ccg atc acc 336
Arg Ile Arg Lys Val Lys Val Glu Phe Trp Pro Cys Ser Pro Ile Thr
cag ggt gac agg gga gtg ggc tcc agt get gtt att tta gat gat aac 384
Gln Gly Asp Arg Gly Val Gly Ser Ser Ala Val Ile Leu Asp Asp Asn
115120125
ttt gta aca aag gcc aca gcc etc acc tat gac cec tat gta aac tac Phe Val Thr Lys Ala Thr Ala Leu Thr Tyr Asp Pro Tyr Val Asn Tyr130135140
tcc tec cgc cat acc ata acc cag ecc toc toc tac cac tcc egg tac
Ser Ser Arg His Thr Ile Thr Gln Pro Phe Ser Tyr His Ser Arg Tyr
ttt acc ccc aaa cct gtc cta gat ttc act att gat tac ttc caa cca
Phe Thr Pro Lys Pro Val Leu Asp Phe Thr Ile Asp Tyr Phe Gln Pro
aac aac aaa aga aac cag ctg tgg ctg aga cta caa act gct gga aat
Asn Asn Lys Arg Asn Gln Leu Trp Leu Arg Leu Gln Thr Ala Gly Asn
180185190
gta gac cac gta ggc ctc ggc act gcg ttc gaa aac agt ata tac gac
Val Asp His Val Gly Leu Gly Thr Ala Phe Glu Asn Ser Ile Tyr Asp
195200205
cag gaa tac aat atc cgt gta acc atg tat gta caa ttc aga gaa ttt
Gln Glu Tyr Asn Ile Arg Val Thr Met Tyr Val Gln Phe Arg Glu Phe
210 215 220
aat ttt aaa gac ccc cca ctt aac cct taa
702
Asn Phe Lys Asp Pro Pro Leu Asn Pro
$\begin{array}{rl}\text { Asn Phe Lys Asp Pro Pro } \\ 225 & 230\end{array}$
$<210>$ SEQ ID NO 26
$<211>$ LENGTH: 233
$<212>$ TYPE: PRT
$<213>$ ORGANISM: TYpe B PWD circovirus
$<400>$ SEQUENCE: 26
Met Thr Tyr Pro Arg Arg Arg Tyr Arg Arg Arg Arg His Arg Pro Arg
1

|  |  |  | 20 |  |  |  |  | 25 |  |  |  |  | 30 |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Arg | His | $\begin{aligned} & \text { Arg } \\ & 35 \end{aligned}$ | Tyr | Arg | $\operatorname{Trp}$ | Arg | $\begin{aligned} & \text { Arg } \\ & 40 \end{aligned}$ | Lys | Asn | Gly | Ile | Phe 45 | Asn | Thr | Arg |
| Leu | $\begin{aligned} & \text { Ser } \\ & 50 \end{aligned}$ | Arg | Thr | Phe | Gly | $\begin{aligned} & \text { Tyr } \\ & 55 \end{aligned}$ | Thr | Val | Lys A | Arg | $\begin{aligned} & \text { Thr } \\ & 60 \end{aligned}$ | Thr | Val | Arg | Thr |
| Pro <br> 65 | Ser | $\operatorname{Trp}$ | Ala | Val | Asp <br> 70 | Met | Met | Arg | Phe | $\begin{aligned} & \text { Asn } \\ & 75 \end{aligned}$ | Ile | Asn | Asp | Phe | $\begin{aligned} & \text { Leu } \\ & 80 \end{aligned}$ |
| Pro | Pro | Gly | Gly | $\begin{aligned} & \text { Gly } \\ & 85 \end{aligned}$ | Ser | Asn | Pro | Arg | $\begin{aligned} & \text { Ser V } \\ & 90 \end{aligned}$ |  | Pro | Phe | Glu | $\begin{aligned} & \text { TYr } \\ & 95 \end{aligned}$ | Tyr |
| Arg | Ile | Arg | $\begin{aligned} & \text { Lys } \\ & 100 \end{aligned}$ | Val | Lys | Val | Glu | $\begin{aligned} & \text { Phe } \\ & 105 \end{aligned}$ | $\text { Trp } P$ | Pro | Cys | Ser | $\begin{aligned} & \text { Pro } \\ & 110 \end{aligned}$ | Ile | Thr |
| Gln | Gly | Asp <br> 115 | Arg | Gly | Val | Gly | $\begin{aligned} & \text { Ser } \\ & 120 \end{aligned}$ | ser | Ala V | Val | Ile | $\begin{aligned} & \text { Leu } \\ & 125 \end{aligned}$ | Asp | Asp | Asn |
| Phe | $\begin{aligned} & \text { Val } \\ & 130 \end{aligned}$ | Thr | Lys | Ala | Thr | $\begin{aligned} & \text { Ala } \\ & 135 \end{aligned}$ | Leu | Thr | Tyr A | Asp | $\begin{aligned} & \text { Pro } \\ & 140 \end{aligned}$ | Tyr | Val | Asn | TYr |
| $\begin{aligned} & \text { Ser } \\ & 145 \end{aligned}$ | Ser | Arg | is | Thr | $\begin{aligned} & \text { Ile } \\ & 150 \end{aligned}$ | Thr | Gln | Pro | Phe | $\begin{aligned} & \text { Ser } \\ & 155 \end{aligned}$ | Tyr | His | Ser | Arg | $\begin{aligned} & \text { TYr } \\ & 160 \end{aligned}$ |
| Phe | Thr | Pro | Lys | $\begin{aligned} & \text { Pro } \\ & 165 \end{aligned}$ | Val | Leu | Asp | Phe | $\begin{aligned} & \text { Thr I } \\ & 170 \end{aligned}$ | Ile | Asp | Tyr | Phe | $\begin{aligned} & \text { Gln } \\ & 175 \end{aligned}$ | Pro |
| Asn | Asn | Lys | Arg <br> 180 | Asn | Gln | Leu | $\operatorname{Trp}$ | $\begin{aligned} & \text { Leu } \\ & 185 \end{aligned}$ | Arg L | Leu | $\mathrm{Gln}$ | Thr | $\begin{aligned} & \text { Ala } \\ & 190 \end{aligned}$ | Gly | Asn |
| Val | Asp | His $195$ | Val | Gly | Leu | Gly | $\begin{aligned} & \text { Thr } \\ & 200 \end{aligned}$ | Ala | Phe | Glu | Asn | $\begin{aligned} & \text { Ser } \\ & 205 \end{aligned}$ | Ile | Tyr | Asp |
| Gln | $\begin{aligned} & \text { Glu } \\ & 210 \end{aligned}$ | Tyr | Asn | Ile | Arg | $\begin{aligned} & \text { Val } \\ & 215 \end{aligned}$ | Thr | Met | Tyr V | Val | $\begin{aligned} & \mathrm{Gln} \\ & 220 \end{aligned}$ | Phe | Arg |  | Phe |
| $\begin{aligned} & \text { Asn } \\ & 225 \end{aligned}$ | Phe | Lys | Asp | Pro | Pro $230$ | Leu | Asn | Pro |  |  |  |  |  |  |  |

$<210>$ SEQ ID NO 27
$<211>$ LENGTH: 315
$<212>$ TYPE: DNA
$<213>$ ORGANISM: TYPe B PWD circovirus
$<220>$ FEATURE:
$<221>$ NAME/KEY: CDS
$<222>$ LOCATION: (1) .. (312)
$<400>$ SEQUENCE: 27
atg gta acc atc coa coa ctt gtt tct agg tgg ttt cca gta tgt ggt Met Val Thr Ile Pro Pro Leu Val Ser Arg Trp Phe Pro Val Cys Gly $\begin{array}{llll}1 & 5 & 10 & 15\end{array}$
ttc cgg gtc tgc aaa att agc agc cca ttt get ttt acc aca ccc agg Phe Arg Val Cys Lys Ile Ser Ser Pro Phe Ala Phe Thr Thr Pro Arg 202530
tgg cec cac aat gac gtg tac att agt ctt cca atc acg ctt ctg cat3540192ttt ccc gct cac ttt caa aag ttc agc cag ccc geg gaa att tct gacPhe Pro Ala His Phe Gln Lys Phe Ser Gln Pro Ala Glu Ile Ser Asp505560aaa cgt tac agg gtg ctg ctc tgc aac ggt cac cag act ccc gct ctc240Lys Arg Tyr Arg Val Leu Leu Cys Asn Gly His Gln Thr Pro Ala Leu 657580caa caa ggt act cac agc agt aga cag gtc act cog ttg tcc ctg aga288


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<210> SEQ ID NO 29
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Type B PWD circovirus
<400> SEQUENCE: 29
Val Asp Met Met Arg Phe Asn Ile Asn Asp Phe Leu Pro Pro Gly
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$<210>$ SEQ ID NO 30
$<211>$ LENGTH: 15
$<212>$ TYPE: PRT
$<213>$ ORGANISM: TYpe B PWD circovirus
$<400>$ SEQUENCE 30
Gln Gly Asp Arg Gly Val Gly Ser Ser Ala Val Ile Leu Asp Asp
$<210>$ SEQ ID NO 31
$<211>$ LENGTH: 15
$<212>$ TYPE: PRT
$<213>$ ORGANISM: Type B PWD circovirus
$<400>$ SEQUENCE : 31
Gly Val Gly Ser Ser Ala Val Ile Leu Asp Asp Asn Phe Val Thr
$<210>$ SEQ ID NO 32
$<211>$ LENGTH: 15
$<212>$ TYPE: PRT
$<213>$ ORGANISN: Type B PWD circovirus
$<400>$ SEQUENCE: 32
Val Asp His Val Gly Leu Gly Thr Ala Phe Glu Asn Ser Ile Tyr

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<210> SEQ ID NO 33
<211> LENGTH: 8
<212> TYPE: DNA
<213> ORGANISM: Type A PWD circovirus
<400> SEQUENCE: 33
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tgtggega 8
<210> SEQ ID NO 34
<211> LENGTH: 8
$<212\rangle$ TYPE: DNA
$<213>$ ORGANISM: TYpe A PWD circovirus
$<400>$ SEQUENCE: 34
agttect 8
$<210>$ SEQ ID NO 35
$<211>$ LENGTH: 20
$<212>$ TYPE: DNA
$<213>$ ORGANISM: TYpe A PWD circovirus
$<400>$ SEQUENCE: 35
<400> SEQUENCE: 35
tcatttagag ggtctttcag 20
$<210\rangle$ SEQ ID NO 36
$<211>$ LENGTH: 8
<212> TYPE: DNA
<213> ORGANISM: Type A PWD circovirus
<400> SEQUENCE: 36

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gtcaacct
```

<210> SEQ ID NO 37
<211> LENGTH: 8
<212> TYPE: DNA
<213> ORGANISM: TYpe A PWD circovirus
<400> SEQUENCE: 37

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gtggttgc 8
\(<210>\) SEQ ID NO 38
\(<211>\) LENGTH: 8
\(<212>\) TYPE: DNA
\(<213>\) ORGANISM: TYpe A PWD circovirus
\(<400>\) SEQUENCE: 38
agcecagg 8
\(<210>\) SEQ ID NO 39
\(<211>\) LENGTH: 8
\(<212>\) TYPE: DNA
\(<213>\) ORGANISM: TYpe A PWD circovirus
\(<400>\) SEQUENCE: 39
ttggetgg 8
\(<210>\) SEQ ID NO 40
\(<211>\) LENGTH: 12
\(<212>\) TYPE: DNA
\(<213>\) ORGANISM: TYpe A PWD circovirus
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<400> SEQUENCE: 40
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<210> SEQ ID NO 41
<211> LENGTH: 12
<212> TYPE: DNA
<213> ORGANISM: Type A PWD circovirus
<400> SEQUENCE: 41
atctcagctc gt 12
<210> SEQ ID NO 42
<211> LENGTH: 12
<212> TYPE: DNA
<213> ORGANISM: Type A PWD circovirus
<400> SEQUENCE: 42
tgtcctcctc tt
<210> SEQ ID NO 43
<211> LENGTH: 8
<212> TYPE: DNA
<213> ORGANISM: Type A PWD circovirus
<400> SEQUENCE: 43
tctctaga
<210> SEQ ID NO 44
<211> LENGTH: 8
<212> TYPE: DNA
<213> ORGANISM: Type A PWD circovirus
<400> SEQUENCE: 44

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tgtaccaa 8
<210> SEQ ID NO 45
<211> LENGTH: 8
<212> TYPE: DNA
\(<213>\) ORGANISM: TYpe A PWD circovirus
\(<400>\) SEQUENCE: 45
tecgtctt 8
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<210> SEQ ID NO 46
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer
<400> SEQUENCE: 46

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20
\(<210>\) SEQ ID NO 47
<211> LENGTH: 20
\(<212>\) TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
\(<223>\) OTHER INFORMATION: Primer
\(<400>\) SEQUENCE: 47
tggaatgtta acgagctgag ..... 20

\(<210>\) SEQ ID NO 48

<211> LENGTH: 20

\(<212>\) TYPE: DNA

\(<213>\) ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 48
ctcgcagcea tettggaatg
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<211> LENGTH: 20
\(<212>\) TYPE: DNA
\(<213>\) ORGANISM: Artificial Sequence
<220> FEATURE:
\(<223>\) OTHER INFORMATION: Primer
\(<400>\) SEQUENCE: 49
agcgegtaat acgactcact 20
<210> SEQ ID NO 50
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
\(<223>\) OTHER INFORMATION: Primer
<400> SEQUENCE: 50
cctgtctact gctgtgagta ccttgt
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<210> SEQ ID NO 51
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

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\(<400>\) SEQUENCE : 51
gcagtagaca ggtcactccg ttgtcc 26
<210> SEQ ID NO 52
<211> LENGTH: 20
<212> TYPE: DNA
\(<213>\) ORGANISM: Artificial Sequence
<220> FEATURE:
\(<223>\) OTHER INFORMATION: Primer
<400> SEQUENCE: 52
tggaatgtta actacctcaa 20
\(<210>\) SEQ ID NO 53
\(<211>\) LENGTH: 23
\(<212>\) TYPE: DNA
\(<213>\) ORGANISM: Artificial Sequence
\(<220>\) FEATURE:
\(<223>\) OTHER INFORMATION: Primer
\(<400>\) SEQUENCE: 53
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<210> SEQ ID NO 54
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer
<400> SEQUENCE: 54
gatggcgccg aaagacgggt atc 23
<210> SEQ ID NO 55
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Type B PWD circovirus
<400> SEQUENCE: 55

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Asn Val Asn Glu Leu Arg Phe Asn Ile Gly Gln Phe Leu Pro Pro
<210> SEQ ID NO 56
<211> LENGTH: 14
\(<212>\) TYPE: PRT
\(<213>\) ORGANISM: Type A PWD circovirus
\(<400>\) SEQUENCE: 56
Thr Ser Asn Gln Arg Gly Val Gly Ser Thr Val Val Ile Leu
<210> SEQ ID NO 57
<211> LENGTH: 15
\(<212>\) TYPE: PRT
<213> ORGANISM: Type A PWD circovirus
<400> SEQUENCE: 57
Arg Gly Val Gly Ser Thr Val Val Ile Leu Asp Ala Asn Phe Val
<210> SEQ ID NO 58
<211> LENGTH: 15
\(<212>\) TYPE: PRT
<213> ORGANISM: Type B PWD circovirus
\(<400>\) SEQUENCE: 58
Phe Thr Ile Asp Tyr Phe Gln Pro Asn Asn Lys Arg Asn Gln Leu
<210> SEQ ID NO 59
<211> LENGTH: 15
\(<212>\) TYPE: PRT
<213> ORGANISM: Type A PWD circovirus
<400> SEQUENCE: 59
Asp Gln Thr Ile Asp Trp Phe Gln Pro Asn Asn Lys Arg Asn Gln
\(<210>\) SEQ ID NO 60
\(<211>\) LENGTH: 15
\(<212>\) TYPE: PRT
\(<213>\) ORGANISN: Type A PWD circovirus
\(<400>\) SEQUENCE: 60
Asn Val Glu His Thr Gly Leu Gly Tyr Ala Leu Gln Asn Ala Thr
\(15010 \quad 15\)
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<210> SEQ ID NO 61
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Type B PWD circovirus
<400> SEQUENCE: 61
His Arg Pro Arg Ser His Leu Gly Gln Ile Leu Arg Arg Arg Pro
1 5 10 10
<210> SEQ ID NO 62
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Type B PWD circovirus
<400> SEQUENCE: 62
Ser His Leu Gly Gln Ile Leu Arg Arg Arg Pro Trp Leu Val His
1 [5 % Arg Pro Trp Leu val Als

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\(<210>\) SEQ ID NO 63
<211> LENGTH: 15
\(<212\rangle\) TYPE: PRT
<213> ORGANISM: Type B PWD circovirus
<400> SEQUENCE: 63
Gln Ile Leu Arg Arg Arg Pro Trp Leu Val His Pro Arg His Arg
<210> SEQ ID NO 64
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: TYpe B PWD circovirus
\(<400>\) SEQUENCE: 64

<210> SEQ ID NO 65
\(<211>\) LENGTH: 15
\(<212>\) TYPE: PRT
<213> ORGANISM: TYpe B PWD circovirus
<400> SEQUENCE: 65
Leu Val His Pro Arg His Arg Tyr Arg Trp Arg Arg Lys Asn Gly
\(15010 \quad 15\)
\(<210>\) SEQ ID NO 66
\(<211>\) LENGTH: 15
\(<212>\) TYPE : PRT
\(<213>\) ORGANISM: TYpe B PWD circovirus
\(<400>\) SEQUENCE: 66
Arg His Arg Tyr Arg Trp Arg Arg Lys Asn Gly Ile Phe Asn Thr
\(<210\rangle\) SEQ ID NO 67
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Type B PWD circovirus
\(<400>\) SEQUENCE: 67
Arg Trp Arg Arg Lys Asn Gly Ile Phe Asn Thr Arg Leu Ser Arg

\begin{tabular}{lcccccc} 
Pro Ser Trp Ala Val Asp Met Met Arg Phe Asn Ile Asn Asp Phe \\
1 & 5 & 10 & 15
\end{tabular}
\(<210>\) SEQ ID NO 75
\(<211>\) LENGTH: 15
\(<212>\) TYPE: PRT
\(<213>\) ORGANISM: Type B PWD circovirus
\(<400>\) SEQUENCE: 75
Arg Phe Asn Ile Asn Asp Phe Leu Pro Pro Gly Gly Gly Ser Asn
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<210> SEQ ID NO 76
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Type B PWD circovirus
<400> SEQUENCE: 76

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Asn Asp Phe Leu Pro Pro Gly Gly Gly Ser Asn Pro Arg Ser Val
210> SEQ ID NO 77
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Type B PWD circovirus
<400> SEQUENCE: 77
\(\begin{array}{ll}\text { Pro Pro Gly Gly Gly Ser Asn Pro Arg Ser Val Pro Phe Glu Tyr } \\ 1 & 5\end{array}\)
<210> SEQ ID NO 78
<211> LENGTH: 15
<212> TYPE: PRT
\(<213>\) ORGANISM: Type B PWD circovirus
<400> SEQUENCE: 78
Gly Ser Asn Pro Arg Ser Val Pro Phe Glu Tyr Tyr Arg Ile Arg
<210> SEQ ID NO 79
<211> LENGTH: 15
\(<212>\) TYPE: PRT
\(<213>\) ORGANISM: Type B PWD circovirus
<400> SEQUENCE: 79

\(<210>\) SEQ ID NO 80
\(<211>\) LENGTH: 15
\(<212>\) TYPE: PRT
\(<213>\) ORGANISM: TYpe B PWD circovirus
\(<400>\) SEOUENCE: 80
Phe Glu Tyr Tyr Arg Ile Arg Lys Val Lys Val Glu Phe Trp Pro
\(<210>\) SEQ ID NO 81
\(<211>\) LENGTH: 15
\(<212>\) TYPE : PRT
\(<213>\) ORGANISM: TYpe B PWD circovirus
\(<400>\) SEQUENCE: 81
Arg Ile Arg Lys Val Lys Val Glu Phe Trp Pro Cys Ser Pro Ile
1
Asn Ile Leu Arg Arg Arg Pro Tyr Leu Val His Pro Ala Phe Arg
\(<210>\) SEQ ID NO 87
\(<211>\) LENGTH: 15
\(<212>\) TYPE: PRT
\(<213>\) ORGANISM: TYpe A PWD circovirus
\(<400>\) SEQUENCE: 87
\begin{tabular}{|c|c|c|c|c|c|c|c|c|c|}
\hline \multicolumn{10}{|c|}{\multirow[b]{4}{*}{\[
5
\]}} \\
\hline & & & & & & & & & \\
\hline & & & & & & & & & \\
\hline & & & & & & & & & \\
\hline
\end{tabular}
```

<210> SEQ ID NO }8
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Type A PWD circovirus

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<400> SEQUENCE: 88
Leu Val His Pro Ala Phe Arg Asn Arg Tyr Arg Trp Arg Arg Lys
\begin{tabular}{l}
5
\end{tabular}

1
\begin{tabular}{lccccc} 
Ala Phe Arg Asn Arg Tyr Arg Trp Arg Arg Lys Thr Gly Ile Phe \\
1 & 5 & 10 & 15
\end{tabular}
\(<210>\) SEQ ID NO 90
\(<211>\) LENGTH: 15
\(<212>\) TYPE: PRT
\(<213>\) ORGANISM: TYpe A PWD circovirus
\(<400>\) SEQUENCE : 90
\begin{tabular}{lcccccc} 
Arg Tyr Arg Trp Arg Arg Lys Thr Gly \\
1 & 5 & 10 & Ale Ane Asn Ser Arg Leu \\
1 & 10
\end{tabular}
\(<210>\) SEQ ID NO 91
\(<211>\) LENGTH: 15
\(<212>\) TYPE : PRT
\(<213>\) ORGANISM: TYpe A PWD circovirus
\(<400>\) SEQUENCE : 91
\begin{tabular}{lcccccc} 
Arg Arg Lys Thr Gly Ile Phe Asn Ser Arg Leu Ser Arg Glu Phe \\
1 & 5 & 10 & 15
\end{tabular}
\(<210>\) SEQ ID NO 92
\(<211>\) LENGTH: 15
\(<212>\) TYPE: PRT
\(<213>\) ORGANISM: TYpe A PWD circovirus
\(<400>\) SEQUENCE: 92

\(<210>\) SEQ ID NO 93
\(<211>\) LENGTH: 15
\(<212>\) TYPE: PRT
\(<213>\) ORGANISM: TYpe A PWD circovirus
\(<400>\) SEQUENCE: 93
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\hline \multicolumn{16}{|l|}{\multirow[t]{2}{*}{\(15010 \quad 15\)}} \\
\hline & & & & & & & & & & & & & & & \\
\hline
\end{tabular}
\(<210>\) SEQ ID NO 94
\(<211>\) LENGTH: 15
\(<212>\) TYPE : PRT
\(<213>\) ORGANISM: TYpe A PWD circovirus
\(<400>\) SEQUENCE: 94

\(<210>\) SEQ ID NO 95
\(<211>\) LENGTH: 15
\(<212>\) TYPE: PRT
\(<213>\) ORGANISM: TYpe A PWD circovirus
<400> SEQUENCE: 95
Leu Thr Ile Arg Gly Gly His Ser Gln Pro Ser Trp Asn Val Asn
1

\(<210>\) SEQ ID NO 99
\(<211>\) LENGTH: 15
\(<212>\) TYPE : PRT
\(<213>\) ORGANISM: TYPe A PWD circovirus
\(<400>\) SEQUENCE : 99
\begin{tabular}{lll} 
Leu Arg Phe Asn Ile Gly Gln Phe Leu Pro Pro Ser Gly Gly Thr \\
1 & 5 & 10
\end{tabular}
\(<210>\) SEQ ID NO 100
\(<211>\) LENGTH: 15
\(<212>\) TYPE: PRT
\(<213>\) ORGANISM: TYpe A PWD circovirus
\(<400>\) SEQUENCE : 100

\(<210>\) SEQ ID NO 101
\(<211>\) LENGTH: 15
\(<212>\) TYPE: PRT
\(<213>\) ORGANISM: TYpe A PWD circovirus
\(<400>\) SEQUENCE : 101

\begin{tabular}{l}
\(<213>\) ORGANISM: TYpe A PWD circovirus \\
\(<400>\) SEQUENCE: 102 \\
Gly Gly Thr Asn Pro Leu Pro Leu Pro Phe Gln Tyr Tyr Arg Ile \\
1 \\
\\
\hline
\end{tabular}
\(<210>\) SEQ ID NO 104
\(<211>\) LENGTH: 15
\(<212>\) TYPE: PRT
\(<213>\) ORGANISM: TYpe A PWD circovirus
\(<400>\) SEQUENCE: 104
Pro Phe Gln Tyr Tyr Arg Ile Arg Lys Ala Lys Tyr Glu Phe Tyr
<210> SEQ ID NO 105
<211> LENGTH: 15
\(<212>\) TYPE: PRT
<213> ORGANISM: Type A PWD circovirus
\(<400>\) SEQUENCE: 105
Tyr Arg Ile Arg Lys Ala Lys Tyr Glu Phe Tyr Pro Arg Asp Pro
5
\(<210>\) SEQ ID NO 106
\(<211>\) LENGTH: 15
\(<212>\) TYPE: PRT
\(<213>\) ORGANISM: TYpe A PWD circovirus
\(<400>\) SEQUENCE: 106
Lys Ala Lys Tyr Glu Phe Tyr Pro Arg Asp Pro Ile Thr
5
\(<210>\) SEQ ID NO 107
\(<211>\) LENGTH: 15
\(<212>\) TYPE: PRT
\(<213>\) ORGANISM: TYpe A PWD circovirus
\(<400>\) SEQUENCE: 107
Glu Phe Tyr Pro Arg Asp Pro Ile Thr Ser Asn Gln Arg Gly Val
\(<210>\) SEQ ID NO 108
\(<211>\) LENGTH: 15
\(<212>\) TYPE: PRT
\(<213>\) ORGANISM: TYpe A PWD circovirus
\(<400>\) SEQUENCE: 108
Arg Asp Pro Ile Thr Ser Asn Gln Arg Gly Val Gly Ser Thr Val
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<212> TYPE: PRT
<213> ORGANISM: Type A PWD circovirus
<400> SEQUENCE: 109

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Thr Ser Asn Gln Arg Gly Val Gly Ser Thr Val Val Ile Leu Asp
\(<210\rangle\) SEQ ID NO 110
<211> LENGTH: 15
\(<212>\) TYPE: PRT
<213> ORGANISM: Type B PWD circovirus
<400> SEQUENCE: 110
Gly Val Gly Ser Ser Ala Val Ile Leu Asp Asp Asn Phe Val Thr
<210> SEQ ID NO 111
\(<211>\) LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: TYpe B PWD circovirus
\(<400>\) SEQUENCE: 111
Ser Ala Val Ile Leu Asp Asp Asn Phe Val Thr Lys Ala Thr Ala
\(<210>S E Q\) ID NO 112
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: TYpe B PWD circovirus
\(<400>\) SEQUENCE: 112
Leu Asp Asp Asn Phe Val Thr Lys Ala Thr Ala Leu Thr Tyr Asp
\(<210>\) SEQ ID NO 113
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: TYpe B PWD circovirus
\(<400>\) SEQUENCE : 113
Phe Val Thr Lys Ala Thr Ala Leu Thr Tyr Asp Pro Tyr Val Asn
\(<210>S E Q\) ID NO 114
<211> LENGTH: 15
\(<212\rangle\) TYPE: PRT
<213> ORGANISM: Type B PWD circovirus
<400> SEQUENCE: 114
Ala Thr Ala Leu Thr Tyr Asp Pro Tyr Val Asn Tyr Ser Ser Arg
\(<210>\) SEQ ID NO 115
<211> LENGTH: 15
\(<212>\) TYPE: PRT
<213> ORGANISM: Type B PWD circovirus
<400> SEQUENCE: 115
Thr Tyr Asp Pro Tyr Val Asn Tyr Ser Ser Arg His Thr Ile Thr
\(<210>\) SEQ ID NO 116
\(<211>\) LENGTH: 15
\(<212>\) TYPE: PRT
\(<213>\) ORGANISM: TYpe B PWD circovirus
\(<400>\) SEQUENCE: 116

\(<210>\) SEQ ID NO 117
\(<211>\) LENGTH: 15
\(<212>\) TYPE: PRT
\(<213>\) ORGANISM: TYpe B PWD circovirus
\(<400>\) SEQUENCE: 117

\(<210>\) SEQ ID NO 118
\(<211>\) LENGTH: 15
\(<212>\) TYPE: PRT
\(<213>\) ORGANISM: TYpe B PWD circovirus
\(<400>\) SEQUENCE: 118

\(<210>\) SEQ ID NO 119
\(<211>\) LENGTH: 15
\(<212>\) TYPE: PRT
\(<213>\) ORGANISM: TYpe B PWD circovirus
\(<400>\) SEQUENCE: 119
\begin{tabular}{|c|c|c|c|}
\hline \multicolumn{4}{|l|}{\multirow[t]{2}{*}{\begin{tabular}{lcccccccccccr} 
Pro Phe Ser Tyr His Ser Arg Tyr Phe Thr Pro Lys Pro Val Leu \\
1 & 5 & 10 & 15
\end{tabular}}} \\
\hline & & & \\
\hline
\end{tabular}
\(<210>\) SEQ ID NO 120
\(<211>\) LENGTH: 15
\(<212>\) TYPE: PRT
\(<213>\) ORGANISM: TYpe B PWD circovirus
\(<400>\) SEQUENCE: 120

\(<210>\) SEQ ID NO 121
\(<211>\) LENGTH: 15
\(<212>\) TYPE: PRT
\(<213>\) ORGANISM: TYpe B PWD circovirus
\(<400>\) SEQUENCE: 121
\begin{tabular}{|c|c|c|c|}
\hline \multicolumn{4}{|l|}{\multirow[t]{2}{*}{}} \\
\hline & & & \\
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\end{tabular}
\(<210>\) SEQ ID NO 122
\(<211>\) LENGTH: 15
\(<212>\) TYPE: PRT
\(<213>\) ORGANISM: TYpe B PWD circovirus
\(<400>\) SEQUENCE: 122

\(<210>\) SEQ ID NO 123
\(<211>\) LENGTH: 15
\(<212>\) TYPE : PRT
\(<213>\) ORGANISM: TYpe B PWD circovirus
\(<400>\) SEQUENCE: 123

\(<210>\) SEQ ID NO 124
\(<211>\) LENGTH: 15
\(<212>\) TYPE: PRT
\(<213>\) ORGANISM: TYpe B PWD circovirus
\(<400>\) SEQUENCE: 124

\(<210>\) SEQ ID NO 125
\(<211>\) LENGTH: 15
\(<212>\) TYPE: PRT
\(<213>\) ORGANISM: TYpe B PWD circovirus
\(<400>\) SEQUENCE: 125

\(<210>\) SEQ ID NO 126
\(<211>\) LENGTH: 15
\(<212>\) TYPE : PRT
\(<213>\) ORGANISM: TYpe B PWD circovirus
\(<400>\) SEQUENCE: 126

\(<210>\) SEQ ID NO 127
\(<211>\) LENGTH: 15
\(<212>\) TYPE: PRT
\(<213>\) ORGANISM: TYpe B PWD circovirus
\(<400>\) SEQUENCE: 127
\begin{tabular}{|c|c|c|c|c|c|}
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\hline \multicolumn{2}{|r|}{\multirow[t]{3}{*}{eu Arg Leu Gln Thr Ala Gly Asn Val Asp His Val Gly Leu
5}} & & & & \\
\hline & & & & & \\
\hline & & & & & \\
\hline & & & & & \\
\hline
\end{tabular}
\(<210>\) SEQ ID NO 128
\(<211>\) LENGTH: 15
\(<212>\) TYPE: PRT
\(<213>\) ORGANISM: TYpe B PWD circovirus
\(<400>\) SEQUENCE: 128
\begin{tabular}{|c|c|c|}
\hline \multicolumn{3}{|l|}{\multirow[t]{4}{*}{Thr Ala Gly Asn Val Asp His Val Gly Leu Gly Thr Ala Phe Glu}} \\
\hline & & \\
\hline & & \\
\hline & & \\
\hline
\end{tabular}
\(<210>\) SEQ ID NO 129
\(<211>\) LENGTH: 15
\(<212>\) TYPE : PRT
\(<213>\) ORGANISM: TYpe B PWD circovirus
\(<400>\) SEQUENCE: 129
\begin{tabular}{|c|c|c|c|c|}
\hline \multicolumn{5}{|l|}{\multirow[t]{5}{*}{\(5-10\) 15}} \\
\hline & & & & \\
\hline & & & & \\
\hline & & & & \\
\hline & & & & \\
\hline
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\(<210>\) SEQ ID NO 130
\(<211>\) LENGTH: 15
\(<212>\) TYPE: PRT
\(<213>\) ORGANISM: TYpe B PWD circovirus
\(<400>\) SEQUENCE: 130
Ala Phe Glu Asn Ser Ile Tyr Asp Gln Glu Tyr Asn Ile Arg Val
\(<210>\) SEQ ID NO 131
\(<211>\) LENGTH: 15
\(<212>\) TYPE : PRT
\(<213>\) ORGANISM: TYpe B PWD circovirus
\(<400>\) SEQUENCE: 1.31
Ser Ile Tyr Asp Gln Glu Tyr Asn Ile Arg Val Thr Met Tyr Val
\(<210>\) SEQ ID NO 132
\(<211>\) LENGTH: 15
\(<212>\) TYPE: PRT
\(<213>\) ORGANISM: TYpe B PWD circovirus
\(<400>\) SEQUENCE: 132
Gln Glu Tyr Asn Ile Arg Val Thr Met Tyr Val Gln Phe Arg Glu
\(<210>\) SEQ ID NO 1.33
\(<211>\) LENGTH: 15
\(<212>\) TYPE: PRT
\(<213>\) ORGANISM: Type B PWD circovirus
\(<400>\) SEQUENCE: 1.33

\(<210>\) SEQ ID NO 134
\(<211>\) LENGTH: 15
\(<212>\) TYPE: PRT
\(<213>\) ORGANISM: Type B PWD circovirus
\(<400>\) SEQUENCE: 134
Met Tyr Val Gln Phe Arg Glu Phe Asn Phe Lys Asp Pro Pro Leu
\(<210>\) SEQ ID NO 1.35
\(<211>\) LENGTH: 15
\(<212>\) TYPE: PRT
\(<213>\) ORGANISM: Type B PWD circovirus
\(<400>\) SEQUENCE : 135
Val Gln Phe Arg Glu Phe Asn Phe Lys Asp Pro Pro Leu Asn Pro
\(<210>\) SEQ ID NO 136
\(<211>\) LENGTH: 15
\(<212>\) TYPE: PRT
\(<213>\) ORGANISN: Type A PWD circovirus
\(<400>\) SEQUENCE: 136
Arg Gly Val Gly Ser Thr Val Val Ile Leu Asp Ala Asn Phe Val
```

<210> SEQ ID NO 137
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Type A PWD circovirus
<400> SEQUENCE: 137
Ser Thr Val Val Ile Leu Asp Ala Asn Phe Val Thr Pro Ser Thr
1 [5 10 10, 15
<210> SEQ ID NO 138
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Type A PWD circovirus
<400> SEQUENCE: 138
Ile Leu Asp Ala Asn Phe Val Thr Pro Ser Thr Asn Leu Ala Tyr

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\(<210>S E Q\) ID NO 139
<211> LENGTH: 15
\(<212\rangle\) TYPE: PRT
<213> ORGANISM: Type A PWD circovirus
<400> SEQUENCE: 139

\(<210>S E Q\) ID NO 140
\(<211>\) LENGTH: 15
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\(<400>\) SEQUENCE: 140
Pro Ser Thr Asn Leu Ala Tyr Asp Pro Tyr Ile Asn Tyr Ser Ser
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\(<210>S E Q\) ID NO 141
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<213> ORGANISM: TYpe A PWD circovirus
\(<400>\) SEQUENCE: 141
Leu Ala Tyr Asp Pro Tyr Ile Asn Tyr Ser Ser Arg His Thr Ile
I

5
\(<210>\) SEQ ID NO 142
\(<211>\) LENGTH: 15
\(<212>\) TYPE: PRT
\(<213>\) ORGANISM: TYpe A PWD circovirus
\(<400>\) SEQUENCE: 142
Pro Tyr Ile Asn Tyr Ser Ser Arg His Thr Ile Arg Gln Pro Phe
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<210> SEQ ID NO 143
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<212> TYPE: PRT
<213> ORGANISM: Type A PWD circovirus
<400> SEQUENCE: 143

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Tyr Ser Ser Arg His Thr Ile Arg Gln Pro Phe Thr Tyr His Ser


\(<210>\) SEQ ID NO 151
\(<211>\) LENGTH: 15
\(<212>\) TYPE: PRT
\(<213>\) ORGANISM: TYpe A PWD circovirus
\(<400>\) SEQUENCE: 151
Pro Asn Asn LYs Arg Asn Gln Leu Trp Leu His Leu Asn Thr His
1
\(<210>\) SEQ ID NO 152
\(<211>\) LENGTH: 15
\(<212>\) TYPE : PRT
\(<213>\) ORGANISM: TYpe A PWD circovirus
\(<400>\) SEQUENCE: 152

\(<210>\) SEQ ID NO 153
\(<211>\) LENGTH: 15
\(<212>\) TYPE : PRT
\(<213>\) ORGANISM: TYpe A PWD circovirus
\(<400>\) SEQUENCE: 153

\(<210>\) SEQ ID NO 154
\(<211>\) LENGTH: 15
\(<212>\) TYPE: PRT
\(<213>\) ORGANISM: TYpe A PWD circovirus
\(<400>\) SEQUENCE: 154
Asn Thr His Thr Asn Val Glu His Thr Gly Leu Gly Tyr Ala Leu
\(<210>\) SEQ ID NO 155
\(<211>\) LENGTH: 15
\(<212>\) TYPE: PRT
\(<213>\) ORGANISM: TYpe A PWD circovirus
\(<400>\) SEQUENCE: 155
Asn Val Glu His Thr Gly Leu Gly Tyr Ala Leu Gln Asn Ala Thr
\(<210>\) SEQ ID NO 156
\(<211>\) LENGTH: 15
\(<212>\) TYPE : PRT
\(<213>\) ORGANISM: TYpe A PWD circovirus
\(<400>\) SEQUENCE: 156

\(<210>\) SEQ ID NO 157
\(<211>\) LENGTH: 15
\(<212>\) TYPE : PRT
\(<213>\) ORGANISM: TYpe A PWD circovirus
\(<400>\) SEQUENCE: 157
Tyr Ala Leu Gln Asn Ala Thr Thr Ala Gln Asn Tyr Val Val Arg
1
10
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Tyr Val Gln Phe Arg Glu Phe Ile Leu Lys Asp Pro Leu Asn Glu \\
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\(<210>\) SEQ ID NO 163
\(<211>\) LENGTH: 1759
\(<212>\) TYPE: DNA
\(<213>\) ORGANISM: TYpe A PWD circovirus
\(<400>\) SEQUENCE: 163
accagcgcac ttcggcagcg gcagcacctc ggcagcgtca gtgaaaatgc caagcaagaa ..... 60
aagcggcceg caaccceata agaggtgggt gttcaccett aataatcctt ccgaggagga ..... 120
gaaaaacaaa atacgggagc ttccaatctc cctttttgat tattttgttt gcggagagga ..... 180
aggtttggaa gagggtagaa ctcetcacct ccaggggttt gegaattttg ctaagaagca ..... 240
gacttttaac aaggtgaagt ggtattttgg tgcccgctgc cacatcgaga aagcgaaagg ..... 300
aaccgaccag cagaataaag aatactgcag taaagaaggc cacatactta tcgagtgtgg ..... 360
agctccgcgg aaccagggga agcgcagcga cctgtctact gctgtgagta cccttttgga ..... 420
gacggggtct ttggtgactg tagccgagca gttccctgta acgtatgtga gaaatttccg ..... 480
cgggctggct gaacttttga aagtgagcgg gaagatgcag aagcgtgatt ggaagacagc ..... 540
tgtacacgtc atagtgggcc cgcccggttg tgggaagagc cagtgggccc gtaattttgc ..... 600
tgagcctagg gacacctact ggaagcctag tagaaataag tggtgggatg gatatcatgg ..... 660
agaagaagtt gttgttttgg atgattttta tggctggtta ccttgggatg atctactgag ..... 720
actgtgtgac cggtatccat tgactgtaga gactaaaggg ggtactgttc cttttttggc ..... 780
ccgcagtatt ttgattacca gcaatcaggc cccccaggaa tggtactcct caactgctgt ..... 840
cccagctgta gaagctctct atcggaggat tactactttg caattttgga agactgctgg ..... 900
agaacaatcc acggaggtac cegaaggceg atttgaagca gtggacccac cetgtgccet ..... 960
tttcccatat aaaataaatt actgagtctt ttttgttatc acatcgtaat ggtttttatt ..... 1020
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ttaccacata attttggget gtggttgcat tttggagcge atagcccagg cetgtgtgct ..... 1140
cgacattggt gtgggtattt aaatggagcc acagctggtt tcttttatta tttgggtgga ..... 1200
accaatcaat tgtttggtcc agctcaggtt tgggggtgaa gtacctggag tggtaggtaa ..... 1260
agggctgcct tatggtgtgg cgggaggagt agttaatata ggggtcatag gccaagttgg ..... 1320
tggagggggt tacaaagttg gcatccaaga taacaacagt ggacccaaca cctctttgat ..... 1380
tagaggtgat ggggtctctg gggtaaaatt catatttagc ctttctaata cggtagtatt ..... 1440
ggaaaggtag gggtaggggg ttggtgccgc ctgagggggg gaggaactgg ccgatgttga ..... 1500
atttcagcta gttaacattc caagatggct gcgagtatcc tccttttatg gtgagtacaa ..... 1560
attctgtaga aaggegggaa ttgaagatac cegtctttog gegccatctg taacggtttc ..... 1620
tgaaggcggg gtgtgccaaa tatggtcttc tccggaggat gtttccaaga tggctgcggg ..... 1680
ggcgggtcct tcttctgcgg taacgcctcc ttggccacgt catcctataa aagtgaaaga ..... 1740
agtgcgctgc tgtagtatt ..... 1759
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<211> LENGTH: 1759

<212> TYPE: DNA

\(<213>\) ORGANISM: Type A PWD circovirus

\(<400>\) SEQUENCE: 164
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aagcggcccg caaccccata agaggtgggt gttcaccctt aataatcctt ccgaggagga ..... 120
gaaaaacaaa atacgggagc ttccaatctc cctttttgat tattttgttt gcggagagga ..... 180
aggtttggaa gagggtagaa ctcctcacct ccaggggttt gctaattttg ctaagaagca ..... 240
gacttttaac aaggtgaagt ggtattttgg tgcccgctgc cacatcgaga aagcgaaagg ..... 300
aaccgaccag cagaataaag aatactgcag taaagaaggc cacatactta tcgagtgtgg ..... 360
agctccgcgg aaccagggga agcgcagcga cctgtctact gctgtgagta cccttttgga ..... 420
gacggggtct ttggtgactg tagccgagca gttccetgta acgtatgtga gaaatttccg ..... 480
cgggctgget gaacttttga aagtgagcgg gaagatgcag aagcgtgatt ggaagacagc ..... 540
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\hline tgagcetagc & gacacctact ggaagcotag & tagaaataag tggtgggatg & gatatcatgg & 660 \\
\hline agaagaagtt & gttgttttgg atgattttta & tggctggtta cettgggatg & atctactgag & 720 \\
\hline actgtgtgac & cggtatccat tgactgtaga & gactaaaggc ggtactgttc & cttttttggc & 780 \\
\hline tcgcagtatt & ttgattacca gcaatcaggc & cccccaggaa tggtactcct & caactgctgt & 840 \\
\hline cccagetgta & gaagctetct atcggaggat & tactactttg caattttgga & agactgctgg & 900 \\
\hline agaacaatca & acggaggtac cogaaggcog & atttgaagca gtggacccac & cotgtgcect & 960 \\
\hline tttcccatat & aaaataaatt actgagtctt & ttttgttatc acatcgtaat & ggtttttatt & 1020 \\
\hline tttatttatt & tagagggtct tttaggataa & ttctctgaa ttgtacataa & atagtcagcc & 1080 \\
\hline ttaccacata & attttgggct gtggttgcat & tttggagcge atagcecagg & cctgtgtgct & 1140 \\
\hline cgacattggt & gtgggtattt aatggagcc & acagctggtt tcttttatta & tttgggtgga & 1200 \\
\hline accattcaat & tgtttggtcc agctcaggtt & tgggggtgaa gtacctggag & tggtaggtaa & 1260 \\
\hline agggctgcet & tatggtgtgg egggaggagt & agttaatata ggggtcatag & gccaagttgg & 1320 \\
\hline tggagggggt & tacaaagttg gcatccaaga & taacaacagt ggacccaaca & cctctttcat & 1380 \\
\hline tagaggtgat & ggggtctctg gggtaaaatt & atatttagc ctttctaata & cggtagtatt & 1440 \\
\hline ggaaaggtag & gggtaggggg ttggtgccge & ctgaggggag gaggaactgg & ccgatgttga & 1500 \\
\hline atctgaggtg & gttaacatgc caagatggct & gegagtatcc tecttttatg & gtgattacaa & 1560 \\
\hline attctttaga & aaggcggcaa ttgaagatac & ccgtctttcg gcgccatctg & taacggtttc & 1620 \\
\hline tgaaggcggg & gtgtgccaaa tatggtcttc & tccggaggat gtttccaaga & tggctgcggg & 1680 \\
\hline ggcgggtcet & tcttctgcgg taacgectcc & ttggccacgt catcctataa & aagtgaaaga & 1740 \\
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\(<210>\) SEQ ID NO 165
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\(<212>\) TYPE : PRT
\(<213>\) ORGANISM: TYpe A PWD circovirus
\(<400>\) SEQUENCE: 165



\(<210>\) SEQ ID NO 167
\(<211>\) LENGTH: 233
\(<212>\) TYPE: PRT
\(<213>\) ORGANISM: Type A PWD circovirus
\(<400>\) SEQUENCE: 167

Phe Ile Leu Lys Asp Pro Leu Asn Lys
\(<210>\) SEQ ID NO 168
\(<211>\) LENGTH: 233
\(<212>\) TYPE : PRT
\(<213>\) ORGANISM: TYpe A PWD circovirus
\(<400>\) SEQUENCE: 168

\(<210>\) SEQ ID NO 169
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\(<212>\) TYPE: PRT
\(<213>\) ORGANISM: TYpe A PWD circovirus
\(<400>\) SEQUENCE: 169


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\(<212>\) TYPE: PRT
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\(<400>\) SEQUENCE: 170

\(<210>\) SEQ ID NO 171
\(<211>\) LENGTH: 15
\(<212>\) TYPE: PRT
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\(<400>\) SEQUENCE: 171

\(<210>\) SEQ ID NO 172
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\(<400>\) SEQUENCE: 172

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\(<211>\) LENGTH: 15
\(<212>\) TYPE: PRT
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\(<400>\) SEQUENCE: 173


\(<210>\) SEQ ID NO 174

<211> LENGTH: 15

<212> TYPE: PRT

\(<213>\) ORGANISM: TYpe B PWD circovirus

<400> SEQUENCE: 174

\(<210>S E Q\) ID NO 175
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<400> SEQUENCE: 175
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\(<210>\) SEQ ID NO 176
<211> LENGTH: 15
\(<212\rangle\) TYPE: PRT
<213> ORGANISM: TYpe B PWD circovirus
\(<400>\) SEQUENCE: 176

\(<210>\) SEQ ID NO 177
\(<211>\) LENGTH: 15
\(<212>\) TYPE : PRT
\(<213>\) ORGANISM: Type B PWD circovirus
\(<400>\) SEQUENCE: 177


We claim:
1. A vaccine comprising a nucleotide sequence of the genome of Porcine circovirus type B , or a homologue or fragment thereof, and an acceptable pharmaceutical or veterinary vehicle, wherein the nucleotide sequence is selected from SEQ ID No. 15 or SEQ ID No. 19.
2. The vaccine of claim 1, wherein the homologue has at least \(80 \%\) sequence identity to SEQ ID No. 15 or SEQ ID No. 19.
3. A vaccine comprising a polypeptide encoded by a nucleotide sequence of the genome of Porcine circovirus type B, or a homologue or fragment thereof, and an acceptable pharmaceutical or veterinary vehicle.
4. The vaccine of claim 3, wherein the homologue has at least \(80 \%\) sequence identity to SEQ ID No. 15 or SEQ ID No. 19.
5. The vaccine of claim \(\mathbf{3}\), wherein the nucleotide sequence is selected from SEQ ID No. 23 or SEQ ID No. 25 , or a homologue or fragment thereof.
6. The vaccine of claim 5 , wherein the homologue has at least \(80 \%\) sequence identity to SEQ ID No. 23 or SEQ ID No. 25.
7. The vaccine of claim 5 , wherein the nucleotide sequence is SEQ ID No. 25.
8. The vaccine of claim 3, wherein the polypeptide has the amino acid sequence of SEQ ID No. 24 or SEQ ID No. 26.
9. The vaccine of claim 8, wherein the polypeptide has the amino acid sequence of SEQ ID No. 26.
10. The vaccine of claim 3 , wherein the homologue has at least \(80 \%\) sequence identity to SEQ ID No. 24 or SEQ ID No. 26.
11. The vaccine of claim 10 , wherein the homologue has at least \(80 \%\) sequence identity to SEQ ID No. 26 .
12. The vaccine of claim 3 , wherein the polypeptide has the amino acid sequence of SEQ ID No. 29, SEQ ID No. 30, SEQ ID No. 31, or SEQ ID No. 32.
13. A vaccine comprising a vector and an acceptable pharmaceutical or veterinary vehicle, the vector comprising a nucleotide sequence of the genome of Porcine circovirus type B, or a homologue or fragment thereof.
14. A vaccine according to claim 13 , further comprising a gene coding for an expression product capable of inhibiting or retarding the establishment or development of a genetic or acquired disease.
15. A vaccine comprising a cell and an acceptable pharmaceutical or veterinary vehicle, wherein the cell is transformed with a nucleotide sequence of the genome of Porcine circovirus type B, or a homologue or fragment thereof.
16. A vaccine comprising a pharmaceutically acceptable vehicle and a single polypetide, wherein the single polypeptide consists of SEQ ID No. 26.
\[
\text { * } \quad * \quad * \quad \% \quad *
\]```


[^0]:    Glu Asn Leu Thr -eu His Pro Thr Lys Leu Ile Leu Asn Glu Ser Asn Tyr Met Asn Met Leu Pro *** Thr Pro Pro Aro *** Phe $* * *$ Ile Aro Gln Tle Thr Cys Ile $* * * * * *$ Pro Asn Leu Pro Pro Asp Lys Phe Asn Phe Glu Arg Phe Gin Val ACA AOT AAT TCC CAA TRC ACC CCC CAG AAA ITT TAA TTT AAG AGA CTT AAC AGG 1035 ACC 1044 ACC CCC 1053 AAA 10621071110 TAT TCA TTA AGG GTT AAG TGG GGG GTC TTT AAA ATT AAA TTC TCT GAA TTG TAC Tyr Ser Leu Arg Val Iys Tro Gly Val Phe Lys Ile Iys Phe Ser Glu Leu Tyr -1e His *** Gly Leu Ser Gly Giy Ser Ieu Lys Leu Asn Ser Leu Asn Cys hr

