



(86) Date de dépôt PCT/PCT Filing Date: 2005/04/05
(87) Date publication PCT/PCT Publication Date: 2005/11/17
(45) Date de délivrance/Issue Date: 2013/12/17
(85) Entrée phase nationale/National Entry: 2006/10/05
(86) N° demande PCT/PCT Application No.: US 2005/011502
(87) N° publication PCT/PCT Publication No.: 2005/107796
(30) Priorités/Priorities: 2004/04/23 (US60/565,214);
2004/12/09 (US60/634,736)

(51) Cl.Int./Int.Cl. *A61K 39/12* (2006.01),
C12N 15/85 (2006.01), *C12N 7/00* (2006.01)

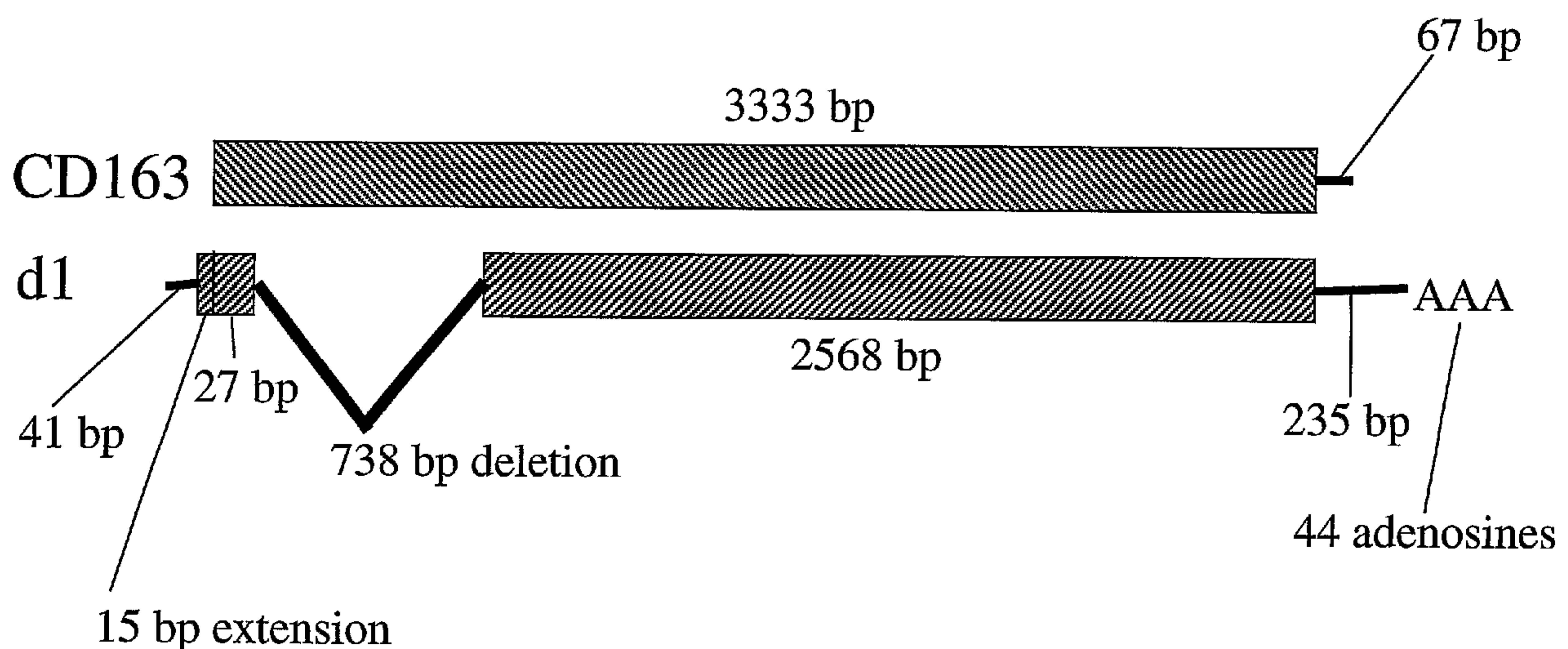
(72) Inventeurs/Inventors:
CALVERT, JAY GREGORY, US;
SHIELDS, SHELLY LYNN, US;
SLADE, DAVID EWELL, US;
WELCH, SIAO-KUN WAN, US

(73) Propriétaire/Owner:
ZOETIS P&U LLC, US

(74) Agent: TORYS LLP

(54) Titre : FACTEUR DE PERMISSIVITE CELLULAIRE POUR VIRUS, ET SON UTILISATION

(54) Title: CELLULAR PERMISSIVITY FACTOR FOR VIRUSES, AND USES THEREOF



(57) Abrégé/Abstract:

The present invention provides methods and compositions related to the generation of host cells permissive for virus growth, particularly Porcine Reproductive and Respiratory Syndrome (PRRS) virus.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
17 November 2005 (17.11.2005)

PCT

(10) International Publication Number
WO 2005/107796 A2

(51) International Patent Classification⁷: **A61K 39/12**,
C12Q 1/68, C12N 7/00, 5/06, 15/86

(21) International Application Number:
PCT/US2005/011502

(22) International Filing Date: 5 April 2005 (05.04.2005)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/565,214 23 April 2004 (23.04.2004) US
60/634,736 9 December 2004 (09.12.2004) US

(71) Applicant (for all designated States except US): **PHARMACIA & UPJOHN COMPANY, LLC** [US/US]; 301 Henrietta Street, Kalamazoo, MI 49001 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **CALVERT, Jay, Gregory** [US/US]; 03400 22nd Street, Otsego, MI 49078 (US). **SHIELDS, Shelly, Lynn** [US/US]; 12200 Pine Lake Road, Plainwell, MI 49080 (US). **SLADE, David, Ewell** [US/US]; 7426 Marfield Street, Portage, MI 49024 (US). **WELCH, Siao-Kun, Wan** [US/US]; 9087 South 6th Street, Kalamazoo, MI 49009 (US).

(74) Agent: **GROLZ, Edward, W.**; Scully, Scott, Murphy & Presser, 400 Garden City Plaza, Garden City, NY 11530 (US).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI,

GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

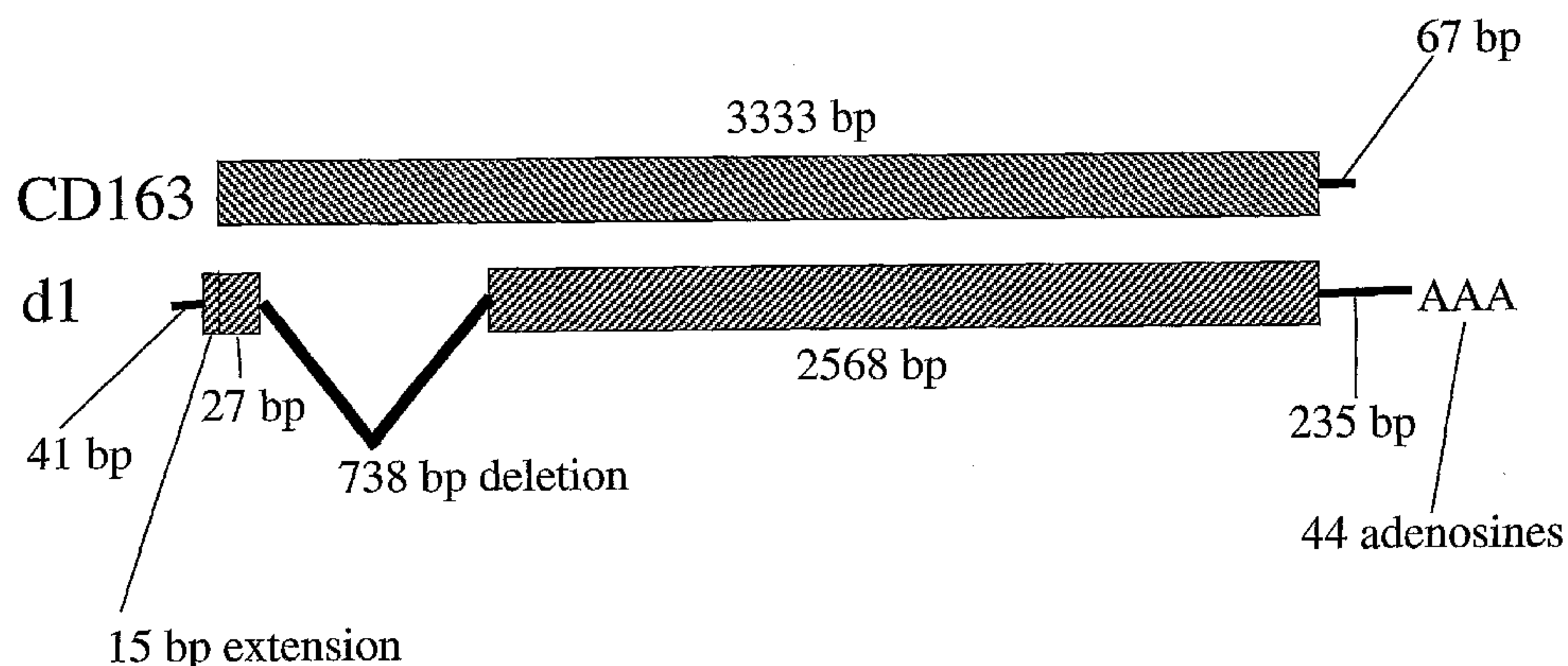
(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

- as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii)) for the following designations AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW, ARIPO patent (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG)
- as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii)) for all designations

[Continued on next page]

(54) Title: CELLULAR PERMISSIVITY FACTOR FOR VIRUSES, AND USES THEREOF



(57) Abstract: The present invention provides methods and compositions related to the generation of host cells permissive for virus growth, particularly Porcine Reproductive and Respiratory Syndrome (PRRS) virus.

WO 2005/107796 A2

WO 2005/107796 A2



— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii)) for all designations*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

Published:

— *without international search report and to be republished upon receipt of that report*

DEMANDES OU BREVETS VOLUMINEUX

**LA PRÉSENTE PARTIE DE CETTE DEMANDE OU CE BREVETS
COMPREND PLUS D'UN TOME.**

CECI EST LE TOME __1__ DE __2__

NOTE: Pour les tomes additionels, veuillez contacter le Bureau Canadien des Brevets.

JUMBO APPLICATIONS / PATENTS

**THIS SECTION OF THE APPLICATION / PATENT CONTAINS MORE
THAN ONE VOLUME.**

THIS IS VOLUME __1__ OF __2__

NOTE: For additional volumes please contact the Canadian Patent Office.

Cellular Permissivity Factor for Viruses, and Uses Thereof FIELD OF THE INVENTION

The present invention provides methods and compositions related to the generation of host cells permissive for virus growth for viruses of the family

5 *Asfarviridae* and *Arteriviridae*.

BACKGROUND OF THE INVENTION

Asfarviridae

Asfarviridae is a family of icosohedral enveloped viruses whose genome consists of a single molecule of linear double-stranded DNA of about 150000–190000 nucleotides
10 long. The name of the family is derived from African Swine Fever And Related Viruses. African Swine Fever Virus (ASFV) is the type species of the Asfivirus genus and is the sole member of the family. Recently, porcine CD163 polypeptide has been surmised by implication to be the cellular receptor for African swine fever virus (ASFV) (Sanchez-Torres et al., 2003)

15 *Arteriviridae*

Viruses of the family of *Arteriviridae* includes equine arteritis virus (EAV), lactate dehydrogenase-elevating virus (LDV) and simian hemorrhagic fever virus (SHFV). The Arterivirus having the greatest economic importance is Porcine Reproductive and Respiratory Syndrome Virus (PRRSV).

20 PRRSV

Porcine Reproductive and Respiratory Syndrome (PRRS) is one of the most economically important diseases of swine. The syndrome appeared almost simultaneously in North American and in Western Europe in the late 1980s, and has since spread to become endemic in the major swine producing nations of Europe,
25 Asia, and the Americas. The etiologic agent of PRRS is a virus that has been designated PRRS virus or PRRSV. For both European and North American PRRS, the disease is characterized by reproductive failure in sows and gilts (late term abortions, still births, and mummies), high mortality among nursery pigs, and respiratory disease in swine of all ages. The disease has been the subject of recent
30 reviews (Mengeling and Lager, 2000; Murtaugh et al., 2002; Nodelijk, 2002; Plagemann, 2003).

In the pig, PRRSV infection is limited to a subset of cells of the monocyte/macrophage lineage. Fully differentiated porcine alveolar macrophage (PAM) cells are the primary target cells for viral replication (Duan et al., 1997a; Duan

et al., 1997b). Immortalization of PAM cells is technically challenging, and when successful, has resulted in cell lines that are not permissive for PRRS virus growth (Weingartl et al., 2002). PRRS virions are specifically bound by macrophages and internalized in clathrin-coated pits by endocytosis. Release from endocytic vesicles requires acidic pH (Nauwynck et al., 1999). Initial binding of virions is mediated by interaction of the viral matrix protein with heparin sulfate glycosaminoglycans (Delputte et al., 2002). Internalization can be facilitated by a 210 or 220 kDa membrane glycoprotein, as incubation of PAM cells with monoclonal antibodies to this polypeptide block infection with PRRS virus (Duan et al., 1998; Wissink et al., 2003). The 210 kDa glycoprotein has recently been identified as sialoadhesin, a member of siglec family of sialic acid binding immunoglobulin-like lectins (Pensaert et al., 2003). Transfection of the non-permissive PK-15 (porcine kidney) cell line with porcine sialoadhesin conferred the ability to internalize PRRSV particles, but there remained an apparent block at the uncoating stage, as virions entered into cellular vesicles but did not undergo nucleocapsid disintegration and vesicle membrane fusion. Viral genes were not expressed, and the transfected PK-15 cells were not rendered permissive for the PRRS virus (Vanderheijden et al., 2003). To our knowledge, transfection with sialoadhesin has not been shown to be sufficient to convert any PRRSV non-permissive cell line to a PRRSV-permissive phenotype.

Apart from primary porcine cells of the monocyte/macrophage lineage, the only other cell type known to be permissive for the growth of PRRSV in cell culture is the immortalized monkey kidney cell line MA-104 (Chladek et al., 1998) and derivatives such as MARC-145 (Kim et al., 1993) and CL-2621. It is not known why this one particular cell line is permissive, yet other mammalian cell lines are not. The PRRS virus binds specifically to a number of different cell types, but does not initiate infection (Kreutz, 1998; Therrien et al., 2000). In MARC-145 cells, the internalization of the virus by endocytosis and subsequent uncoating in low pH vesicles seems to mimic similar events in PAM cells (Kreutz and Ackermann, 1996). However, a number of monoclonal antibodies that bind to porcine sialoadhesin fail to detect a homologous protein on the surface of MARC-145 cells (Duan et al., 1998; Wissink et al., 2003), suggesting that MARC-145 cells may use a divergent member of the same protein family or a different receptor altogether.

Current PRRSV vaccines are propagated on simian cell lines, which is a potentially dangerous activity. The use of simian cell lines for vaccine production has the potential to introduce primate viruses of significance into swine lines intended for xenotransplant purposes. Because swine are being increasingly explored as a source of xenotransplanted organs for humans, the introduction of primate cell lines to swine populations may ultimately pose a risk to humans receiving xenotransplanted organs. Thus, it would be prudent to avoid the use of simian cell lines in swine vaccine preparations. It would be therefore desirable to identify or generate non-simian cells or cell lines capable of supporting PRRSV replication. Towards this goal, it is essential to identify the gene product(s) which may be responsible for conferring the permissivity for PRRSV replication as seen in certain simian cells lines as well as PAM cells. Once such gene products are identified, non-permissive cells might be rendered permissive by transfection of the essential gene into them, thereby affording a wider array of production lines for a vaccine.

One lab has reported that the tetraspanin protein CD151 from MARC-145 cells, when transfected into non-permissive BHK-21 cells, confers permissivity to the PRRS virus (Kapil and Shanmukhappa, 2003; Shanmukhappa and Kapil, 2001). This observation has yet to be confirmed by an independent lab.

We describe here an unrelated polypeptide, which when transfected into non-permissive cells, confers permissivity to the PRRS virus.

References Cited

Chladek, D. W., Harris, L. L., and Gorcyca, D. E. Method of growing and attenuating a viral agent associated with mystery swine disease. Boehringer Ingelheim Animal Health, Inc. 677,585[US 5,840,563], 1-24. 11-24-1998. USA. 7-9-1996.
Ref Type: Patent

Dea,S., Gagnon,C.A., Mardassi,H., Pirzadeh,B., and Rogan,D. (2000). Current knowledge on the structural proteins of porcine reproductive and respiratory syndrome (PRRS) virus: comparison of the North American and European isolates [Review]. Arch. Virol. 145, 659-688.

Delputte,P.L., Vanderheijden,N., Nauwynck,H.J., and Pensaert,M.B. (2002). Involvement of the matrix protein in attachment of porcine reproductive and respiratory syndrome virus to a heparinlike receptor on porcine alveolar macrophages. J. Virol. 76, 4312-4320.

- Duan,X., Nauwynck,H.J., and Pensaert,M.B. (1997a). Effects of origin and state of differentiation and activation of monocytes/macrophages on their susceptibility to porcine reproductive and respiratory syndrome virus (PRRSV). *Arch. Virol.* *142*, 2483-2497.
- 5 Duan,X., Nauwynck,H.J., and Pensaert,M.B. (1997b). Virus quantification and identification of cellular targets in the lungs and lymphoid tissues of pigs at different time intervals after inoculation with porcine reproductive and respiratory syndrome virus (PRRSV). *Vet. Microbiol.* *56*, 9-19.
- 10 Duan,X.B., Nauwynck,H.J., Favoreel,H.W., and Pensaert,M.B. (1998). Identification of a putative receptor for porcine reproductive and respiratory syndrome virus on porcine alveolar macrophages. *J. Virol.* *72*, 4520-4523.
- Graversen,J.H., Madsen,M., and Moestrup,S.K. (2002). CD163: a signal receptor scavenging haptoglobin-hemoglobin complexes from plasma. [Review] [19 refs]. *International Journal of Biochemistry & Cell Biology* *34*, 309-314.
- 15 Gronlund J. Vitved L. Lausen M. Skjodt K. Holmskov U. Cloning of a novel scavenger receptor cysteine-rich type I transmembrane molecule (M160) expressed by human macrophages. *Journal of Immunology* *165*(11):6406-6415, 2000.
- 20 Kapil, S. and Shanmukhappa, K. Host susceptibility factor(s) for porcine reproductive and respiratory syndrome virus and uses in swine breeding, as a target for antiviral compounds, and development of a non-simian recombinant cell line for propagation of the virus. none. US 2003/0186236 A1, 1-45. 10-2-2003. USA. 1-28-2002.
Ref Type: Patent
- 25 Kim,H.S., Kwang,J., Yoon,I.J., Joo,H.S., and Frey,M.L. (1993). Enhanced replication of porcine reproductive and respiratory syndrome (PRRS) virus in a homogenous subpopulation of MA-104 cell line. *Arch. Virol.* *133*, 477-483.
- Kreutz,L.C. (1998). Cellular membrane factors are the major determinants of porcine reproductive and respiratory syndrome virus tropism. *Virus Res.* *53*, 121-128.
- 30 Kreutz,L.C. and Ackermann,M.R. (1996). Porcine reproductive and respiratory syndrome virus enters cells through a low pH-dependent endocytic pathway. *Virus Res.* *42*, 137-147.
- Mengeling,W.L. and Lager,K.M. (2000). A brief review of procedures and potential problems associated with the diagnosis of porcine reproductive and respiratory syndrome. *Veterinary Research* *31*, 61-69.
- 35 Meulenbergh,J.J.M. (2000). PRRSV, the virus. *Veterinary Research* *31*, 11-21.
- Murtaugh,M.P., Xiao,Z.G., and Zuckermann,F. (2002). Immunological responses of swine to porcine reproductive and respiratory syndrome virus infection [Review]. *Viral Immunology* *15*, 533-547.
- 40 Nauwynck,H.J., Duan,X., Favoreel,H.W., Van Oostveldt,P., and Pensaert,M.B. (1999). Entry of porcine reproductive and respiratory syndrome virus into porcine alveolar macrophages via receptor-mediated endocytosis. *J. Gen. Virol.* *80*, 297-305.

- Nodelijk, G. (2002). Porcine Reproductive and Respiratory Syndrome (PRRS) with special reference to clinical aspects and diagnosis - A review [Review]. *Vet. Quart.* 24, 95-100.
- 5 Pensaert, M., Nauwynck, H., and Vanderheijden, N. Nucleic acid encoding polypeptide involved in cellular entrance of the PRRS virus. Akzo Nobel N.V. and Universiteit Gent. WO 03/010200 A2, 1-24. 2-6-2003. 7-18-2002.
Ref Type: Patent
- 10 Philippidis, P., Mason, J.C., Evans, B.J., Nadra, I., Taylor, K.M., Haskard, D.O., and Landis, R.C. (2004). Hemoglobin scavenger receptor CD163 mediates interleukin-10 release and heme oxygenase-1 synthesis - Antiinflammatory monocyte-macrophage responses in vitro, in resolving skin blisters in vivo, and after cardiopulmonary bypass surgery. *Circulation Research* 94, 119-126.
- Plagemann, P.G.W. (2003). Porcine reproductive and respiratory syndrome virus: Origin hypothesis. *Emerging Infectious Diseases* 9, 903-908.
- 15 Ritter, M., Buechler, C., Langmann, T., and Schmitz, G. (1999). Genomic organization and chromosomal localization of the human CD163 (M130) gene: a member of the scavenger receptor cysteine-rich superfamily. *Biochemical & Biophysical Research Communications* 260, 466-474.
- 20 Sanchez-Torres, C., Gomez-Puertas, P., Gomez-del-Moral, M., Alonso, F., Escribano, J.M., Ezquerro, A., and Dominguez, J. (2003). Expression of porcine CD163 on monocytes/macrophages correlates with permissiveness to African swine fever infection. *Arch. Virol.* 148, 2307-2323.
- 25 Shanmukhappa, K. and Kapil, S. (2001). Cloning and identification of MARC-145 cell proteins binding to 3'UTR and partial nucleoprotein gene of porcine reproductive and respiratory syndrome virus. *Adv. Exp. Med. Biol.* 494, 641-646.
- Snijder, E.J. and Meulenbergh, J.J.M. (2001). Arteriviruses. In *Fields Virology*, D.M.Knipe, P.M.Howley, D.E.Griffin, M.A.Martin, R.A.Lamb, B.Roizman, and S.E.Straus, eds. (Philadelphia: Lippincott Williams & Wilkins), pp. 1205-1220.
- 30 Therrien, D., St Pierre, Y., and Dea, S. (2000). Preliminary characterization of protein binding factor for porcine reproductive and respiratory syndrome virus on the surface of permissive and non-permissive cells. *Arch. Virol.* 145, 1099-1116.
- 35 Vanderheijden, N., Delputte, P.L., Favoreel, H.W., Vandekerckhove, J., Van Damme, J., van Woensel, P.A., and Nauwynck, H.J. (2003). Involvement of sialoadhesin in entry of porcine reproductive and respiratory syndrome virus into porcine alveolar macrophages. *J. Virol.* 77, 8207-8215.
- Weingartl, H.M., Sabara, M., Pasick, J., van Moorlehem, E., and Babiuk, L. (2002). Continuous porcine cell lines developed from alveolar macrophages - Partial characterization and virus susceptibility. *J. Virol. Methods* 104, 203-216.
- 40 Wissink, E.H.J., van Wijk, H.A.R., Pol, J.M.A., Godeke, G.J., van Rijn, P.A., Rottier, P.J.M., and Meulenbergh, J.J.M. (2003). Identification of porcine alveolar

macrophage glycoproteins involved in infection of porcine respiratory and reproductive syndrome virus. Arch. Virol. 148, 177-187.

SUMMARY OF THE INVENTION

The invention includes a method of facilitating infection of one or more cells
5 by a virus that is selected from the group consisting of *Arteriviridae* and *Asfarviridae*,
which comprises the step of directing increased expression of a CD163 polypeptide
within said cell. In a preferred embodiment the CD163 is membrane bound. In one
embodiment the virus is selected from the group consisting of *Arteriviridae*. In a
preferred embodiment the virus is PRRSV. In another embodiment said virus is
10 equine arteritis virus (EAV). In yet another embodiment said virus is African Swine
Fever virus (ASFV).

Increased expression of a CD163 polypeptide may be accomplished by
methods such as introduction of exogenous nucleic acids encoding CD163
polypeptides such methods include but are not limited to transfection, electroporation
15 and fusion with a carrier of a polynucleotide comprising a polynucleotide encoding a
CD163 polypeptide. Increased expression may also be accomplished by induction of
expression of endogenous CD163 by chemical treatment.

The method may render previously non PRRSV-permissive cells PRRS
permissive. The method may also include rendering one or more cells that previously
20 did not express a CD163 polypeptide into cells that are induced to express a CD 163
polypeptide.

The cells in a preferred embodiment are animal cells. They may be vertebrate
or invertebrate cells. The cells may be mammalian. The cells or cell line may be an
insect cell line. The cells may be BHK21 cells. The cells may be derived from
25 porcine kidney cells. The cells or cell line may be derived from feline kidney cells.
The cells or cell line may be but are not limited to BHK-21, NLST-1, NLFK-1, Vero
or RL cells. The PRRSV may be or the European or North American genotype.

As noted above, increased expression of a CD163 polypeptide may be
accomplished by methods which include but are not limited to: transfection,
30 electroporation and fusion with a carrier of a polynucleotide comprising a
polynucleotide encoding a CD163 polypeptide. Any CD163 polypeptides are
contemplated. Those containing a transmembrane region are preferred. Exemplary

CD163 polypeptides are selected from the group consisting of the polynucleotides listed below.

One such polynucleotide comprises a polynucleotide encoding a polypeptide having at least 70% 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%,
5 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 93%, 94%, 95%, 96% 97% 98%, 99%, identity with SEQ ID NO: 2.

One such polynucleotide comprises a polynucleotide encoding a polypeptide differing from SEQ ID NO: 2 by no more than 20 conservative amino acid substitutions.

10 One such polynucleotide comprises a polynucleotide encoding a polypeptide differing from SEQ ID NO: 2 by no more than 10 conservative amino acid substitutions.

One such polynucleotide comprises a polynucleotide encoding a polypeptide comprising SEQ ID NO: 2.

15 One such polynucleotide comprises a polynucleotide with the sequence set forth in SEQ ID NO:1

One such polynucleotide comprises a polynucleotide encoding a polypeptide that has at least 99% identity to a polypeptide set forth in SEQ ID NO: 14

One such polynucleotide comprises a polynucleotide encoding a polypeptide
20 differing from SEQ ID NO: 14 by no more than 15 conservative amino acid substitutions.

One such polynucleotide comprises a polynucleotide encoding a polypeptide differing from SEQ ID NO: 14 by no more than 10 conservative amino acid substitutions.

25 One such polynucleotide comprises a polynucleotide encoding a polypeptide comprising SEQ ID NO: 14.

One such polynucleotide comprises a polynucleotide with the sequence set forth in SEQ ID NO: 13.

One such polynucleotide comprises a polynucleotide encoding a polypeptide
30 differing from SEQ ID NO: 24 by no more than 2 conservative amino acid substitutions.

One such polynucleotide comprises a polynucleotide encoding a polypeptide comprising SEQ ID NO: 24.

One such polynucleotide comprises a polynucleotide with the sequence set forth in SEQ ID NO: 23.

One such polynucleotide comprises a polynucleotide encoding a polypeptide having at least 96% 97% 98%, or 99%, identity to a polypeptide set forth in SEQ ID
5 NO: 27.

One such polynucleotide comprises a polynucleotide encoding a polypeptide differing from SEQ ID NO: 27 by no more than 20 conservative amino acid substitutions.

One such polynucleotide comprises a polynucleotide encoding a polypeptide
10 differing from SEQ ID NO: 27 by no more than 10 conservative amino acid substitutions.

One such polynucleotide comprises a polynucleotide encoding a polypeptide comprising SEQ ID NO: 27.

One such polynucleotide comprises a polynucleotide with the sequence set
15 forth in SEQ ID NO: 26.

One such polynucleotide comprises a polynucleotide encoding a polypeptide that has at least 98%, 99% identity to a polypeptide set forth in SEQ ID NO: 32

One such polynucleotide comprises a polynucleotide encoding a polypeptide differing from SEQ ID NO: 32 by no more than 15 conservative amino acid
20 substitutions.

One such polynucleotide comprises a polynucleotide encoding a polypeptide differing from SEQ ID NO: 32 by no more than 10 conservative amino acid substitutions.

One such polynucleotide comprises a polynucleotide encoding a polypeptide
25 comprising SEQ ID NO: 32.

One such polynucleotide comprises a polynucleotide with the sequence set forth in SEQ ID NO: 31.

One such polynucleotide comprises a polynucleotide encoding a polypeptide that has at least 95%, 96%, 97%, 98%, 99% identity to a polypeptide set forth in SEQ
30 ID NO: 34

One such polynucleotide comprises a polynucleotide encoding a polypeptide differing from SEQ ID NO: 34 by no more than 15 conservative amino acid substitutions.

One such polynucleotide comprises a polynucleotide encoding a polypeptide differing from SEQ ID NO: 34 by no more than 10 conservative amino acid substitutions.

One such polynucleotide comprises a polynucleotide encoding a polypeptide comprising SEQ ID NO: 34.

One such polynucleotide comprises a polynucleotide with the sequence set forth in SEQ ID NO: 33.

One such polynucleotide comprises a polynucleotide encoding a polypeptide that has at least 95%, 96%, 97%, 98%, 99% identity to a polypeptide set forth in SEQ ID NO: 36

One such polynucleotide comprises a polynucleotide encoding a polypeptide differing from SEQ ID NO: 36 by no more than 15 conservative amino acid substitutions.

One such polynucleotide comprises a polynucleotide encoding a polypeptide differing from SEQ ID NO: 36 by no more than 10 conservative amino acid substitutions.

One such polynucleotide comprises a polynucleotide encoding a polypeptide comprising SEQ ID NO: 36.

One such polynucleotide comprises a polynucleotide with the sequence set forth in SEQ ID NO: 35.

One such polynucleotide comprises a polynucleotide encoding a polypeptide that has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity to a polypeptide set forth in SEQ ID NO: 42

One such polynucleotide comprises a polynucleotide encoding a polypeptide differing from SEQ ID NO: 42 by no more than 15 conservative amino acid substitutions.

One such polynucleotide comprises a polynucleotide encoding a polypeptide differing from SEQ ID NO: 42 by no more than 10 conservative amino acid substitutions.

One such polynucleotide comprises a polynucleotide encoding a polypeptide comprising SEQ ID NO: 42.

One such polynucleotide comprises a polynucleotide with the sequence set forth in SEQ ID NO: 41.

One such polynucleotide comprises a polynucleotide encoding a polypeptide that has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity to a polypeptide set forth in SEQ ID NO: 44

One such polynucleotide comprises a polynucleotide encoding a polypeptide
5 differing from SEQ ID NO: 44 by no more than 15 conservative amino acid substitutions.

One such polynucleotide comprises a polynucleotide encoding a polypeptide differing from SEQ ID NO: 44 by no more than 10 conservative amino acid substitutions.

10 One such polynucleotide comprises a polynucleotide encoding a polypeptide comprising SEQ ID NO: 44.

One such polynucleotide comprises a polynucleotide with the sequence set forth in SEQ ID NO: 43

One such polynucleotide comprises a polynucleotide encoding a polypeptide
15 that has at least 95%, 96%, 97%, 98%, 99% identity to a polypeptide set forth in SEQ ID NO: 46

One such polynucleotide comprises a polynucleotide encoding a polypeptide differing from SEQ ID NO: 46 by no more than 15 conservative amino acid substitutions.

20 One such polynucleotide comprises a polynucleotide encoding a polypeptide differing from SEQ ID NO: 46 by no more than 10 conservative amino acid substitutions.

One such polynucleotide comprises a polynucleotide encoding a polypeptide comprising SEQ ID NO: 46.

25 One such polynucleotide comprises a polynucleotide with the sequence set forth in SEQ ID NO: 45.

One such polynucleotide comprises a polynucleotide encoding a polypeptide that has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity to a polypeptide set forth in SEQ ID NO: 48

30 One such polynucleotide comprises a polynucleotide encoding a polypeptide differing from SEQ ID NO: 48 by no more than 15 conservative amino acid substitutions.

One such polynucleotide comprises a polynucleotide encoding a polypeptide differing from SEQ ID NO: 48 by no more than 10 conservative amino acid substitutions.

One such polynucleotide comprises a polynucleotide encoding a polypeptide comprising SEQ ID NO: 48.

One such polynucleotide comprises a polynucleotide with the sequence set forth in SEQ ID NO: 47.

The method of facilitating infection described above may further comprise the step of producing a culture of virus.

The invention further comprises the culture isolated by the method described above.

Any of the methods described above may further comprise the step of producing a PRRS or other viral vaccine. The vaccine may be killed or live attenuated.

The invention also comprises a cell or cell line wherein the ability of one or more cells to be infected by a virus selected from the group consisting of *Arteriviridae* and *Asfarviridae* has been modified by directing increased expression of a CD163 polypeptide within said cells.

In a preferred embodiment the CD163 polypeptide comprises a transmembrane region. In one embodiment the virus is selected from the group consisting of *Arteriviridae*. In a preferred embodiment the virus is PRRSV. In another embodiment said virus is equine arteritis virus (EAV). In yet another embodiment said virus is African Swine Fever virus (ASFV).

The cell or cell line of the invention may have been previously PRRSV non-permissive and is rendered PRRSV permissive by directing increased expression of a CD163 polypeptide within said cell or cell line.

The cell or cell line of the invention includes cells or cell lines that did not express a CD163 polypeptide and is induced to express a CD 163 polypeptide.

The cells in a preferred embodiment are animal cells. They may be vertebrate or invertebrate cells. The cells may be mammalian. The cell or cell line may be an insect cell or cell line. The cells may be BHK21 cells. The cells may be derived from porcine kidney cells. The cell or cell line may be derived from feline kidney cells.

The cells may be, but are not limited to, BHK-21, NLST-1, NLFK-1, Vero or RL cells. The PRRSV may be North American or European.

The invention includes a method for measuring the propensity of a test cell or cell line to allow infection by a virus selected from the group consisting of

5 *Arteriviridae* and *Asfarviridae* comprising:

a) providing a sample containing nucleic acids from the test cell or cell line;

b) determining the amount of polynucleotide encoding a CD163 polypeptide or its complement in said sample;

wherein an increased amount of polynucleotide encoding a CD163 polypeptide

10 relative to a control sample derived from a control cell or cell line known not to support the growth of said virus indicates a propensity of the test cell or cell line to support the replication of said virus.

In one embodiment the virus is selected from the group consisting of *Arteriviridae*. In a preferred embodiment the virus is PRRSV. In another embodiment

15 said virus is equine arteritis virus (EAV). In yet another embodiment said virus is African Swine Fever virus (ASFV).

The amount of polynucleotide encoding a CD163 polypeptide may be determined by hybridization.

The amount of polynucleotide encoding a CD163 polypeptide may be

20 determined by PCR.

The invention also includes a method for measuring the propensity of a test cell or cell line to allow infection by a virus selected from the group consisting of *Arteriviridae* and *Asfarviridae* comprising:

(a) providing a sample containing polypeptides from the test cell or cell line;

25 (b) determining the amount of CD163 polypeptide in said sample;

wherein an increased amount of a CD163 polypeptide relative to a control sample derived from a control cell or cell line known not to support the growth of said virus indicates a propensity of the test cell or cell line to support the replication of said virus.

30 In one embodiment the virus is selected from the group consisting of *Arteriviridae*. In a preferred embodiment the virus is PRRSV. In another embodiment said virus is equine arteritis virus (EAV). In yet another embodiment said virus is African Swine Fever virus (ASFV).

In one embodiment the determining is accomplished by contacting a CD163 polypeptide with an antibody specific for the CD163 polypeptide, under conditions wherein the antibody binds the CD163 polypeptide.

The invention includes a method for measuring the propensity of a pig to be
5 infected by a virus selected from the group consisting of *Arteriviridae* and *Asfarviridae* comprising:

- a) providing a sample containing nucleic acids from the pig to be tested;
- b) determining the amount of polynucleotide encoding a CD163 polypeptide or its complement in said sample;

10 wherein an increased amount of polynucleotide encoding a CD163 polypeptide relative to a control sample derived from a pig known to be resistant to said virus infection indicates a propensity of the pig to be tested to be infected by said virus.

In one embodiment the virus is selected from the group consisting of *Arteriviridae*. In a preferred embodiment the virus is PRRSV. In another embodiment
15 said virus is equine arteritis virus (EAV). In yet another embodiment said virus is African Swine Fever virus (ASFV).

In one embodiment the determining is accomplished by hybridization. In another embodiment the determining is accomplished by PCR.

The invention also includes a method for measuring the propensity of a pig to
20 be infected by a virus selected from the group consisting of *Arteriviridae* and *Asfarviridae* comprising:

- (a) providing a sample containing polypeptides from pig to be tested;
- (b) determining the amount of CD163 polypeptide in said sample;

wherein an increased amount of a CD163 polypeptide relative to a control sample
25 derived from a pig known to be resistant to said virus infection indicates a propensity of the pig to be tested to be infected by said virus.

In one embodiment the virus is selected from the group consisting of *Arteriviridae*. In a preferred embodiment the virus is PRRSV. In another
embodiment said virus is equine arteritis virus (EAV). In yet another embodiment
30 said virus is African Swine Fever virus (ASFV).

In one embodiment the determining is accomplished by contacting a CD163 polypeptide with an antibody specific for the CD163 polypeptide, under conditions wherein the antibody binds the CD163 polypeptide.

The invention also includes an isolated polypeptide wherein the polypeptide is selected from the group consisting of the polypeptides described below.

Therefore the invention also includes an isolated polypeptide having at least 70% 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%,
5 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 93%, 94%, 95%, 96% 97% 98%, 99%, identity with SEQ ID NO: 2.

One such polypeptide is a polypeptide differing from SEQ ID NO: 2 by no more than 20 conservative amino acid substitutions.

One such polypeptide is a polypeptide differing from SEQ ID NO: 2 by no
10 more than 10 conservative amino acid substitutions.

One such polypeptide comprises SEQ ID NO: 2.

Therefore the invention also includes an isolated polypeptide having at least 99%, identity to a polypeptide set forth in SEQ ID NO: 14

One such polypeptide is a polypeptide differing from SEQ ID NO: 14 by no
15 more than 15 conservative amino acid substitutions.

One such polypeptide is a polypeptide differing from SEQ ID NO: 14 by no more than 10 conservative amino acid substitutions.

One such polypeptide comprises SEQ ID NO: 14.

Therefore the invention also includes an isolated polypeptide differing from
20 SEQ ID NO: 24 by no more than 2 conservative amino acid substitutions.

One such polypeptide comprises SEQ ID NO: 24.

Therefore the invention also includes an isolated polypeptide having at least 96% 97% 98%, or 99%, identity to a polypeptide set forth in SEQ ID NO: 27.

One such polypeptide is a polypeptide differing from SEQ ID NO: 27 by no
25 more than 20 conservative amino acid substitutions.

One such polypeptide is a polypeptide differing from SEQ ID NO: 27 by no more than 10 conservative amino acid substitutions.

One such polypeptide is a polypeptide comprising SEQ ID NO: 27.

Therefore the invention also includes an isolated polypeptide having at least
30 98%, 99% identity to a polypeptide set forth in SEQ ID NO: 32

One such polypeptide is a polypeptide differing from SEQ ID NO: 32 by no more than 15 conservative amino acid substitutions.

One such polypeptide is a polypeptide differing from SEQ ID NO: 32 by no more than 10 conservative amino acid substitutions.

One such polypeptide is a polypeptide comprising SEQ ID NO: 32.

Therefore the invention also includes an isolated polypeptide having at least
5 95%, 96%, 97%, 98%, 99% identity to a polypeptide set forth in SEQ ID NO: 34

One such polypeptide is a polypeptide differing from SEQ ID NO: 34 by no more than 15 conservative amino acid substitutions.

One such polypeptide is a polypeptide differing from SEQ ID NO: 34 by no more than 10 conservative amino acid substitutions.

10 One such polypeptide is a polypeptide comprising SEQ ID NO: 34.

Therefore the invention also includes an isolated polypeptide having at least 95%, 96%, 97%, 98%, 99% identity to a polypeptide set forth in SEQ ID NO: 36

One such polypeptide is a polypeptide differing from SEQ ID NO: 36 by no more than 15 conservative amino acid substitutions.

15 One such polypeptide is a polypeptide differing from SEQ ID NO: 36 by no more than 10 conservative amino acid substitutions.

One such polypeptide is a polypeptide comprising SEQ ID NO: 36.

The invention also includes an isolated polypeptide having at least 95%, 96%, 97%, 98%, 99% identity to a polypeptide set forth in SEQ ID NO: 38

20 One such polypeptide is a polypeptide differing from SEQ ID NO: 38 by no more than 15 conservative amino acid substitutions.

One such polypeptide is a polypeptide differing from SEQ ID NO: 38 by no more than 10 conservative amino acid substitutions.

One such polypeptide is a polypeptide comprising SEQ ID NO: 40

25 Therefore the invention also includes an isolated polypeptide having at least 95%, 96%, 97%, 98%, 99% identity to a polypeptide set forth in SEQ ID NO: 40

One such polypeptide is a polypeptide differing from SEQ ID NO: 40 by no more than 15 conservative amino acid substitutions.

30 One such polypeptide is a polypeptide differing from SEQ ID NO: 40 by no more than 10 conservative amino acid substitutions.

One such polypeptide is a polypeptide comprising SEQ ID NO: 40.

. Therefore the invention also includes an isolated polypeptide having at 90%. 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity to a polypeptide set forth in SEQ ID NO: 42

One such polypeptide is a polypeptide differing from SEQ ID NO: 42 by no
5 more than 15 conservative amino acid substitutions.

One such polypeptide is a polypeptide differing from SEQ ID NO: 42 by no more than 10 conservative amino acid substitutions.

One such polypeptide is a polypeptide comprising SEQ ID NO: 42.

Therefore the invention also includes an isolated polypeptide having at least
10 90%. 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity to a polypeptide set forth in SEQ ID NO: 44

One such polypeptide is a polypeptide differing from SEQ ID NO: 44 by no more than 15 conservative amino acid substitutions.

One such polypeptide is a polypeptide differing from SEQ ID NO: 44 by no
15 more than 10 conservative amino acid substitutions.

One such polypeptide is a polypeptide comprising SEQ ID NO: 44.

Therefore the invention also includes an isolated polypeptide having at least 90%. 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity to a polypeptide set forth in SEQ ID NO: 46

20 One such polypeptide is a polypeptide differing from SEQ ID NO: 46 by no more than 15 conservative amino acid substitutions.

One such polypeptide is a polypeptide differing from SEQ ID NO: 46 by no more than 10 conservative amino acid substitutions.

One such polypeptide is a polypeptide comprising SEQ ID NO: 46.

25 Therefore the invention also includes an isolated polypeptide having at least 90%. 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity to a polypeptide set forth in SEQ ID NO: 48

One such polypeptide is a polypeptide differing from SEQ ID NO: 48 by no more than 15 conservative amino acid substitutions.

30 One such polypeptide is a polypeptide differing from SEQ ID NO: 48 by no more than 10 conservative amino acid substitutions.

One such polynucleotide comprises a polynucleotide encoding a polypeptide comprising SEQ ID NO: 48.

The invention also includes an isolated CD163 polynucleotide wherein said polynucleotide is selected from the group consisting of the polynucleotides enumerated below.

Therefore the invention also includes an isolated polynucleotide comprising:

- 5 (a) a polynucleotide sequence set forth in SEQ ID NOs: 1 or 5
- (b) a polynucleotide that encodes a polypeptide that has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 93%, 94%, 95%, 96% 97% 98%, or 99%, identity and or similarity to a polypeptide set forth in SEQ ID NO: 2
- 10 (c) a polynucleotide encoding a polypeptide of SEQ ID: 2,
- (d) a polynucleotide which is the complement of any of (a), (b) or (c).

Therefore the invention also includes an isolated polynucleotide comprising:

- (a) a polynucleotide sequence set forth in SEQ ID NOs: 12 or 13
- (b) a polynucleotide that encodes a polypeptide that has at least 99%, identity and or
- 15 similarity to a polypeptide set forth in SEQ ID NO: 14
- (c) a polynucleotide encoding a polypeptide of SEQ ID: 14,
- (d) a polynucleotide which is the complement of any of (a), (b) or (c).

Therefore the invention also includes an isolated polynucleotide comprising:

- (a) a polynucleotide sequence set forth in SEQ ID NOs: 22 or 23
- 20 (b) a polynucleotide encoding a polypeptide of SEQ ID: 24,
- (c) a polynucleotide which is the complement of any of (a) or (b).

Therefore the invention also includes an isolated polynucleotide comprising:

- (a) a polynucleotide sequence set forth in SEQ ID NOs: 25 or 26
- (b) a polynucleotide that encodes a polypeptide that has at least 96% 97% 98%, or
- 25 99%, identity and or similarity to a polypeptide set forth in SEQ ID NO: 27
- (c) a polynucleotide encoding a polypeptide of SEQ ID: 27,
- (d) a polynucleotide which is the complement of any of (a), (b) or (c).

Therefore the invention also includes an isolated polynucleotide comprising:

- (a) polynucleotide sequence set forth in SEQ ID NOs: 30 or 31
- 30 (b) a polynucleotide that encodes a polypeptide that has at least 98% or 99%, identity and or similarity to a polypeptide set forth in SEQ ID NO: 32
- (c) a polynucleotide encoding a polypeptide of SEQ ID: 32,
- (d) a polynucleotide which is the complement of any of (a), (b) or (c).

Therefore the invention also includes an isolated polynucleotide comprising:

- (a) polynucleotide sequence set forth in SEQ ID NOs: 33
- (b) a polynucleotide that encodes a polypeptide that has at least 95% 96%, 97%, 98% or 99%, identity and or similarity to a polypeptide set forth in SEQ ID NO: 34
- 5 (c) a polynucleotide encoding a polypeptide of SEQ ID: 34,
- (d) a polynucleotide which is the complement of any of (a), (b) or (c).

Therefore the invention also includes an isolated polynucleotide comprising:

- (a) polynucleotide sequence set forth in SEQ ID NOs: 35
- (b) a polynucleotide that encodes a polypeptide that has at least 95% 96%, 97%, 98% or
- 10 99%, identity and or similarity to a polypeptide set forth in SEQ ID NO: 36
- (c) a polynucleotide encoding a polypeptide of SEQ ID: 36,
- (d) a polynucleotide which is the complement of any of (a), (b) or (c).

Therefore the invention also includes an isolated polynucleotide comprising:

- (a) polynucleotide sequence set forth in SEQ ID NOs: 37
- 15 (b) a polynucleotide that encodes a polypeptide that has at least 95% 96%, 97%, 98% or 99%, identity and or similarity to a polypeptide set forth in SEQ ID NO: 38
- (c) a polynucleotide encoding a polypeptide of SEQ ID: 38,
- (d) a polynucleotide which is the complement of any of (a), (b) or (c).

Therefore the invention also includes an isolated polynucleotide comprising:

- 20 (a) polynucleotide sequence set forth in SEQ ID NOs: 39
- (b) a polynucleotide that encodes a polypeptide that has at least 95% 96%, 97%, 98% or 99%, identity and or similarity to a polypeptide set forth in SEQ ID NO: 40
- (c) a polynucleotide encoding a polypeptide of SEQ ID: 40,
- (d) a polynucleotide which is the complement of any of (a), (b) or (c).

25 Therefore the invention also includes an isolated polynucleotide comprising:

- (a) polynucleotide sequence set forth in SEQ ID NOs: 41
- (b) a polynucleotide that encodes a polypeptide that has at least 95% 96%, 97%, 98% or 99%, identity and or similarity to a polypeptide set forth in SEQ ID NO: 42
- (c) a polynucleotide encoding a polypeptide of SEQ ID: 42,
- 30 (d) a polynucleotide which is the complement of any of (a), (b) or (c).

Therefore the invention also includes an isolated polynucleotide comprising:

- (a) polynucleotide sequence set forth in SEQ ID NOs: 43

64680-1737

(b) a polynucleotide that encodes a polypeptide that has at least 95% 96%, 97%, 98% or 99%, identity and or similarity to a polypeptide set forth in SEQ ID NO: 44

(c) a polynucleotide encoding a polypeptide of SEQ ID: 44,

(d) a polynucleotide which is the complement of any of (a), (b) or (c).

5 Therefore the invention also includes an isolated polynucleotide comprising:

(a) polynucleotide sequence set forth in SEQ ID NOs: 45

(b) a polynucleotide that encodes a polypeptide that has at least 90%, 91%, 92%, 93%, 94%, 95% 96%, 97%, 98% or 99%, identity and or similarity to a polypeptide set forth in SEQ ID NO: 46

10 (c) a polynucleotide encoding a polypeptide of SEQ ID: 46,

(d) a polynucleotide which is the complement of any of (a), (b) or (c).

The invention also includes an isolated polynucleotide comprising:

(a) polynucleotide sequence set forth in SEQ ID NOs: 47

(b) a polynucleotide that encodes a polypeptide that has at least 90%, 91%, 92%, 93%,
15 94%, 95% 96%, 97%, 98% or 99%, identity and or similarity to a polypeptide set forth in SEQ ID NO: 48

(c) a polynucleotide encoding a polypeptide of SEQ ID: 49,

(d) a polynucleotide which is the complement of any of (a), (b) or (c).

20 Therefore the invention also includes a CD163 polypeptide in which the transmembrane region is deleted.

Therefore the invention also includes a polynucleotide encoding a CD163 polypeptide in which the transmembrane region is deleted.

64680-1737

In another aspect, the invention relates to a method of facilitating production of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) from a culture of vertebrate cells, comprising the steps of: (a) providing a recombinant vertebrate cell transfected with an exogenous polynucleotide that
5 encodes a mammalian CD163 polypeptide having a transmembrane domain and at least 70% sequence identity to SEQ ID NO:14, so that expression of CD163 polypeptide in said cell is increased; (b) contacting a culture of said cell with PRRSV virus under conditions which permit infection of the cells and growth of the virus; and (c) recovering virus from said culture.

10 In addition to the foregoing, the invention includes, as an additional aspect, all embodiments of the invention narrower in scope in any way than the variations specifically mentioned above.

Brief Description of the Sequence Listings

SEQ ID NO:1 cDNA sequence encoding porcine susCD163v1

15 **SEQ ID NO:2** predicted amino acid sequence of porcine susCD163v1

SEQ ID NO:3 cDNA sequence, Genbank accession number AJ311716

SEQ ID NO:4 predicted amino acid sequence derived from Genbank accession number AJ311716

SEQ ID NO:5 cDNA sequence of susCD163v1 containing flanking (non-coding)
20 sequence.

- SEQ ID NO: 6-11---**primer sequences
- SEQ ID NO:12 ---**cDNA sequence encoding porcine susCD163v2 containing flanking (non-coding) sequence.
- SEQ ID NO:13 --**cDNA sequence encoding porcine susCD163v2
- 5 **SEQ ID NO:14 ---**predicted amino acid sequence of porcine susCD163v2
- SEQ ID NO:15-16---**primer sequences
- SEQ ID NO:17---**cDNA sequence encoding human CD163v2 containing flanking (non-coding) sequence.
- SEQ ID NO:18---** cDNA sequence encoding human CD163v2
- 10 **SEQ ID NO:19---** predicted amino acid sequence of human CD163v2
- SEQ ID NO:20-21---**primer sequences
- SEQ ID NO:22---**cDNA sequence encoding murine CD163v2 containing flanking (non-coding) sequence.
- SEQ ID NO:23---** cDNA sequence encoding murine CD163v2
- 15 **SEQ ID NO:24---** predicted amino acid sequence of murine CD163v2
- SEQ ID NO:25---**cDNA sequence encoding murine CD163v3 containing flanking (non-coding) sequence.
- SEQ ID NO:26---** cDNA sequence encoding murine CD163v3
- SEQ ID NO:27---** predicted amino acid sequence of murine CD163v3
- 20 **SEQ ID NO:28-29---**primer sequences
- SEQ ID NO:30---**cDNA sequence encoding MARC-145 CD163v3 containing flanking (non-coding) sequence.
- SEQ ID NO:31---** cDNA sequence encoding MARC-145 CD163v3
- SEQ ID NO:32---** predicted amino acid sequence of MARC-145 CD163v3
- 25 **SEQ ID NO:33--**cDNA sequence encoding Vero cell CD163v2 transcript
- SEQ ID NO:34--** predicted amino acid sequence of Vero cell CD163v2
- SEQ ID NO:35--** cDNA sequence encoding Vero cell CD163v3 transcript
- SEQ ID NO:36--** predicted amino acid sequence of Vero cell CD163v3
- SEQ ID NO:37--** cDNA sequence encoding Vero cell CD163v4 transcript
- 30 **SEQ ID NO:38--** predicted amino acid sequence of Vero cell CD163v4
- SEQ ID NO:39--** cDNA sequence encoding Vero cell CD163v5 transcript
- SEQ ID NO:40--** predicted amino acid sequence of Vero cell CD163v5
- SEQ ID NO:41--** cDNA sequence encoding Vero cell CD163v6 transcript

SEQ ID NO:42-- predicted amino acid sequence of Vero cell CD163v6

SEQ ID NO:43-- cDNA sequence encoding Vero cell CD163v7 transcript

SEQ ID NO:44-- predicted amino acid sequence of Vero cell CD163v7

SEQ ID NO:45-- cDNA sequence encoding canine CD163v2 transcript.

5 **SEQ ID NO:46--** predicted amino acid sequence of canine CD163v2

SEQ ID NO:47-- cDNA sequence encoding canine CD163v3 transcript.

SEQ ID NO:48-- predicted amino acid sequence of canine CD163v3

Brief Description of the Drawings

10 **Figure 1** Schematic comparison of susCD163v1 with AJ311716

Figure 2 Amino acid Sequence alignment of susCD163v1 (SEQ ID NO: 2) with AJ311716 (SEQ ID NO: 4)

Figure 3 Nucleotide Sequence alignment of susCD163v1 with AJ311716

15 **Figure 4** Generation of DNA fragments and ligation to place CD163 directly behind the RSV promoter. Plasmids were digested with either *DraIII* or *DrdI*, followed by a blunting reaction with Klenow enzyme. After clean up, the plasmids were digested with *NotI*. Gel purification yielded DNA fragments that were subsequently ligated utilizing the cohesive *NotI* termini. Promoters from RSV (pRSV) and SV40 (pSV40) are indicated with arrows.

20 **Figure 5** Map of pCDNA3.1 Directional V5/His/TOPO cloning vector

Figure 6 Three BHK/CMV/v1 cell lines, #3, #5, and #12 and a non-permissive BHK cell line were infected with PRRSV isolate P129 and stained with SDOW17-FITC. Panel A shows a non-permissive BHK21 cell clone. Panel B shows BHK/CMV/v1 clone #3. Panel C shows BHK/CMV/v1 clone #5. Panel D shows BHK/CMV/v1
25 clone #12.

Figure 7 Three BHK/RSV/v1 cell lines, #2, #3, and #4 were infected with PRRSV isolate P129 and stained with SDOW17-FITC. Panel A shows BHK/RSV/v1 clone #2. Panel B shows BHK/RSV/v1 clone #3. Panel C shows BHK/RSV/v1 clone #4.

Figure 8 Feline kidney cell lines stably expressing porcine CD163v1, showing
30 PRRSV plaques. Cell lines NLFK-CMV-susCD163v1-G4F and NLFK-CMV-susCD163v1-G4L, both at passage 4, were infected with the P129 isolate of North American PRRSV and incubated for 6 days. Monolayers were fixed with 80% acetone and stained with monoclonal antibody SDOW17-FITC. Phase contrast

microscopy (right) shows localized regions of viral CPE (plaques), while FA detection (left) shows co-localized viral nucleocapsid antigen.

Figure 9 Four FK/RSV/v1 cell lines, #1, #2, #3, and #4 were infected with PRRSV isolate P129 and stained with monoclonal antibody SDOW17-FITC. Panel A shows
5 FK/RSVv1 #1 cell clone. Panel B shows FK/RSV/v1 clone #2. Panel C shows FK/RSV/v1 clone #3. Panel D shows FK/RSV/v1 #4.

Figure 10 PK-CMV-susCD163v1-A10 cells at passage 19, infected with PRRSV isolate P129. Left: The monolayer was fixed with 80% acetone and stained with FITC-conjugated monoclonal antibody SDOW17 (Rural Technologies Inc), which is
10 specific for PRRSV nucleocapsid. Right: The same well under bright field illumination, showing cell distribution.

Figure 11 BHK-CMVScript-susCD163v2-A9 at passage 17 infected with PRRSV isolate P129. The monolayer was fixed with 80% acetone and stained with FITC-conjugated monoclonal antibody SDOW17 (Rural Technologies Inc), which is
15 specific for PRRSV nucleocapsid.

Figure 12 Three representative examples of the BHK/RSV/v2 cell lines. The cells were infected with PRRSV isolate P129 and subsequently stained with SDOW17-FITC. Panel A shows cell line BHK/RSV/v2 #1, panel B shows cell line BHK/RSV/v2 #34, and panel C shows cell line BHK/RSV/v2 #47.

Figure 13 FK-cDNA3.1D-humCD163v2-A6 at passage 15 infected with PRRSV isolate P129. The monolayer was then fixed with 80% acetone and stained with FITC-conjugated monoclonal antibody SDOW17 (Rural Technologies Inc), which is
20 specific for PRRSV nucleocapsid.

Figure 14 The amount of progeny PRRSV produced by four recombinant cell lines stably expressing susCD163v1, and by MARC-145 cells, was determined in a growth
25 curve experiment using the NVSL 94-3 isolate of PRRSV. Samples harvested at 12-hour intervals were titrated on MARC-145 cell monolayers.

Figure 15 Flow cytometry analysis of PRRSV infection in the presence of CD163 specific antibody. BHK-21 cells expressing MARC-145 CD163 from transient
30 transfection were incubated with either CD163 specific antibody or normal goat IgG (NGS) and infected with a GFP-expressing PRRSV. Each data point represents the results of triplicate wells.

Figure 16 Flow cytometry analysis of PRRSV infection in the presence of CD163 specific antibody. NLFK cells stably expressing human CD163 were incubated with either CD163 specific antibody or normal goat IgG (NGS) and infected with a GFP expressing PRRSV. At 24 hours post infection the percentage of GFP expressing infected cells was determined. Each data point represents the result from a single well of cells

Figure 17. Graphical depiction of six alternative splicing variants of CD163 mRNA recovered from Vero cells. The six variants differ in the presence or absence of three exons, designated E6, E105, and E83. Exons E6 and E105 have lengths that are multiples of three, and therefore do not result in a change in reading frame when absent. In contrast, the absence of E83 results in a shifted reading frame and an alternative amino acid sequence at the carboxy terminus of the protein (indicated by a hatched pattern in the figure). The hydrophobic transmembrane (TM) region is encoded within E105.

Figure 18. PK-RSVScript-susCD163v2 #9 cells infected with PRRSV isolate P129. Undiluted supernatant from PRRSV isolate P201 infected PAMs was used to infect PK-RSVScript-susCD163v2 #9 cells. After two days of incubation the cells were fixed and stained with monoclonal antibody SDOW17 as described in Example 11.

Figure 19. FK-RSVScript-susCD163v2 #51 cells infected with PRRSV isolate P129. Undiluted supernatant from PRRSV isolate P201 infected PAMs was used to infect FK-RSVScript-susCD163v2 #51 cells. Two days post infection the cells were acetone fixed and stained with monoclonal antibody SDOW17 as described in Example 11.

Figure 20. Infection of PK-RSVScript-susCD163v2 clone #9 cells with PRRSV isolate P201. Panel A shows a monolayer of cells infected with PRRSV P201 at passage 1, twenty-four hours post infection. Panel B shows a monolayer of cells 2 days post infection with cell free supernatant PRRSV P201 at passage 10.

Figure 21. NLFK parent cells and one subclone of FK-cDNA3.1D-humCD163v2-A6 were examined for the CD163 expression. Cells were fixed in 80% acetone and reacted with Goat anti-human CD163 (R&D System at 1:200) for one hour following by washing with PBS. For visualization, donkey anti-Goat IgG conjugated with FITC (Bioscience Inc at 1:100) were used. No specific fluorescence was detected in the

NLFK parent cells as shown in Figure 21A. The majority of the FK.A6.A2 subclone showed good fluorescent staining indicating the presence of CD163 (Figure 21B).

DETAILED DESCRIPTION OF THE INVENTION

5 General Definitions

Cells and cell lines can be either virus permissive” or “virus non-permissive”. For example, a cell or cell line that is virus permissive is capable of allowing virus infection, subsequent replication and virus production. A cell or cell line that is virus non-permissive is incapable of allowing virus infection, subsequent replication and virus production. A cell line that is already somewhat permissive may be rendered more permissive by the methods of the invention.

Arteriviridae refers to a family of enveloped, positive-stranded RNA viruses belonging to the order *Nidovirales*. The family includes lactate dehydrogenase-elevating virus (LDV) of mice, equine arteritis virus (EAV), simian hemorrhagic fever virus (SHFV), and PRRSV.

Asfarviridae is a family of icosohedral, enveloped viruses whose genomes consist of single molecules of linear double-stranded DNA about 150000–190000 nucleotides long. The name of the family is derived from African Swine Fever And Related Viruses. African Swine Fever Virus (ASFV) is the type species of the Asfivirus genus and is the sole member of the family.

The term “PRRSV” or PRRS virus refers to both European and North American PRRS virus genotypes. Within each genotype, isolates typically share 85% or higher nucleotide identity. Between genotypes, however, the level of sequence identity is only about 60%.

The PRRS virus is a member of the family *Arteriviridae*. The genome of the arteriviruses is single-stranded RNA of positive polarity between 12 and 16 kb in length, capped at the 5' end and polyadenylated at the 3' end. Over two-thirds of the genome is dedicated to open reading frames (ORFs) 1a and 1b, which encode the non-structural functions of the virus. ORF1b is an extension of ORF1a, and is the result of a ribosomal frameshift. ORFs 1a and 1b are translated directly from the genomic RNA. These large polypeptide products are cleaved by viral proteases to yield 12 or 13 discrete smaller peptides. The remaining ORFs, which encode viral structural proteins, are expressed from a series of 3' co-terminal subgenomic RNAs (sgRNAs).

The sgRNAs are produced by discontinuous transcription of negative-stranded RNA, such that a common 5' leader sequence becomes fused to each transcript. The major structural proteins are the nucleocapsid (N, encoded by ORF7), the matrix protein (M, encoded by ORF6), and the major envelope glycoprotein (GP5, encoded by ORF5).

5 The remaining proteins, GP4 (ORF4), GP3 (ORF3), GP2 (ORF2a), and E (ORF2b) are minor structural components of the virion, whose functions have not yet been elucidated. The molecular biology of PRRSV has been the subject of recent review articles (Dea et al., 2000; Meulenberg, 2000; Snijder and Meulenberg, 2001).

As used herein, the term "CD163 polypeptide" means a protein encoded by a mammalian CD163 gene, including allelic variants containing conservative or non-conservative changes. A cDNA sequence that encodes a porcine CD163 polypeptide has been reported (Genbank accession number AJ311716). A murine CD163 polypeptide has also been reported (Genbank access number AF274883), as well as multiple human variants, exemplified by Genbank access numbers AAH51281 and
15 CAA80543. We report herein polynucleotides that encode porcine, human, murine, canine, and african green monkey CD163 polypeptides and which comprise the sequences set forth in SEQ ID NO: 1, 5, 12 13, 17, 18, 22, 23, 25, 26, 30, 31 33, 35, 37, 39, 41, 42, 43, 45 and 47. A "CD163 polypeptide" is a member of the scavenger receptor cysteine-rich (SRCR) family of transmembrane glycoproteins, and is thought
20 to be expressed exclusively on monocytes and macrophages. One identified role of CD163 is to inhibit oxidative tissue damage following hemolysis by consuming hemoglobin:haptoglobin complexes by endocytosis. The subsequent release of interleukin-10 and synthesis of heme oxygenase-1 results in antiinflammatory and cytoprotective effects (Philippidis et al., 2004; Graversen et al., 2002). The human
25 CD163 gene spans 35 kb on chromosome 12, and consists of 17 exons and 16 introns. A number of isoforms of the CD163 polypeptide, including membrane bound, cytoplasmic and secreted types, are known to be generated by alternative splicing (Ritter et al., 1999). Isoforms that comprise a transmembrane domain are particularly preferred.

30 A transmembrane domain is characterized by a polypeptide segment of a larger sequence that is exposed on both sides of a membrane. The cytoplasmic and extracellular domains are separated by at least one membrane-spanning segment that traverses the hydrophobic environment of the lipid bilayer. The membrane- spanning

segment is composed of amino acid residues with nonpolar side chains, usually in the form of an alpha helix. Segments that contain about 20-30 hydrophobic residues are long enough to span a membrane as an alpha helix, and they can often be identified by means of a hydropathy plot. The predicted transmembrane domain of SEQ ID NO:2
5 and 14 are indicated by bolding in the specification. To determine whether other CD163 sequences contain a similar sequence feature is easily determined by inspection of the sequence or hydropathy plots. SEQ ID NOS: 37-40 are representative of variant CD163 proteins which do not contain a transmembrane domain and their encoding nucleic acids.

10 As used hereinafter, "polynucleotide" generally refers to any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. "Polynucleotides" include, without limitation, single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, RNA that is mixture of single-
15 and double-stranded regions, and hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, "polynucleotide" refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The term "polynucleotide" also includes DNAs or RNAs containing one or more modified bases and DNAs or RNAs
20 with backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications may be made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically or metabolically modified forms of polynucleotides as typically found in nature, as well as the chemical forms of DNA and RNA
25 characteristic of viruses and cells. "Polynucleotide" also embraces relatively short polynucleotides, often referred to as oligonucleotides.

As used hereinafter, "polypeptide" refers to any peptide or protein comprising amino acids joined to each other by peptide bonds or modified peptide bonds.

"Polypeptide" refers to both short chains, commonly referred to as peptides,
30 oligopeptides or oligomers, and to longer chains, generally referred to as proteins. Polypeptides may contain amino acids other than the 20 gene-encoded amino acids. "Polypeptides" include amino acid sequences modified either by natural processes, such as post-translational processing, or by chemical modification techniques which

are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature.

Modifications may occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated
5 that the same type of modification may be present to the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched and branched cyclic polypeptides may result from post-translation natural processes or may be made by
10 synthetic methods. Modifications or modified forms include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of
15 covalent cross-links, formation of cystine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination
20 (see, for instance, *Proteins-Structure and Molecular Properties*, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York, 1993; Wold, F., *Post-translational Protein Modifications: Perspectives and Prospects*, pgs. 1-12 in *Postranslational Covalent Modification of Proteins*, B. C. Johnson, Ed., Academic Press, New York, 1983; Seifter et al., "Analysis for protein modifications and nonprotein cofactors", *Meth Enzymol* (1990) 182:626-646 and Rattan et al., "Protein
25 Synthesis: Post-translational Modifications and Aging", *Ann NY Acad Sci* (1992) 663:4842).

As used hereinafter, "isolated" means altered by the hand of man from the natural state. If an "isolated" composition or substance occurs in nature, it has been
30 changed or removed from its original environment, or both. For example, a polynucleotide or a polypeptide naturally present in a living animal is not "isolated," but the same polynucleotide or polypeptide separated from the coexisting materials of its natural state is "isolated", as the term is employed herein. Therefore "isolated" as

used herein and as understood in the art, whether referring to “isolated” polynucleotides or polypeptides, is taken to mean separated from the original cellular environment in which the polypeptide or nucleic acid is normally found. As used herein therefore, by way of example only, a transgenic animal or a recombinant cell
 5 line constructed with a polynucleotide of the invention makes use of the “isolated” nucleic acid. Specifically excluded from the definition of isolated polynucleotides of the invention are entire isolated chromosomes from native host cells from which the polynucleotide was originally derived.

In the disclosure to follow we will often make use of the term “identity” or
 10 similarity as applied to the amino acid sequences of polypeptides. Percent amino acid sequence “identity” with respect to polypeptides is defined herein as the percentage of amino acid residues in the candidate sequence that are identical with the residues in the target sequences after aligning both sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any
 15 conservative substitutions as part of the sequence identity. Percent sequence identity is determined by conventional methods. For example, BLASTP 2.2.6 [Tatusova TA and TL Madden, “BLAST 2 sequences- a new tool for comparing protein and nucleotide sequences.” (1999) FEMS Microbiol Lett. 174:247-250.]

Briefly, as noted above, two amino acid sequences are aligned to optimize the
 20 alignment scores using a gap opening penalty of 10, a gap extension penalty of 0.1, and the “blosum62” scoring matrix of Henikoff and Henikoff (Proc. Nat. Acad. Sci. USA 89:10915-10919. 1992).

The percent identity is then calculated as:

$$\frac{\text{Total number of identical matches}}{[\text{length of the longer sequence} + \text{number of gaps introduced into the longer sequence to align the two sequences}]} \times 100$$

25

Percent sequence “similarity” (often referred to as “homology”) with respect to
 30 a polypeptide of the invention is defined herein as the percentage of amino acid residues in the candidate sequence that are identical with the residues in the target sequences after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity (as described above), and also considering any conservative substitutions as part of the sequence identity.

Total number of identical matches and conservative substitutions x

100

[length of the longer sequence +number of gaps introduced into
the longer sequence to align the two sequences]

5

Amino acids can be classified according to physical properties and contribution to secondary and tertiary protein structure. A conservative substitution is recognized in the art as a substitution of one amino acid for another amino acid that has similar properties.

10 Exemplary conservative substitutions are set out in Table 1, 2, and 3 below.

Table 1
Conservative Substitutions I

15

	SIDE CHAIN	
	CHARACTERISTIC	AMINO ACID
	Aliphatic	
	Non-polar	G A P I L V
20	Polar - uncharged	C S T M N Q
	Polar - charged	D E K R
	Aromatic	H F W Y
25	Other	N Q D E

Alternatively, conservative amino acids can be grouped as described in Lehninger, [Biochemistry, Second Edition; Worth Publishers, Inc. NY:NY (1975), pp.71-77] as set out in Table 2, immediately below

30

Table 2
Conservative Substitutions II

<u>SIDE CHAIN</u>		
5	<u>CHARACTERISTIC</u>	<u>AMINO ACID</u>
	Non-polar (hydrophobic)	
	A. Aliphatic:	A L I V P
	B. Aromatic:	F W
10	C. Sulfur-containing:	M
	D. Borderline:	G
	Uncharged-polar	
	A. Hydroxyl:	S T Y
	B. Amides:	N Q
15	C. Sulfhydryl:	C
	D. Borderline:	G
	Positively Charged (Basic):	K R H
	Negatively Charged (Acidic):	D E

20 As still another alternative, exemplary conservative substitutions are set out in Table 3, immediately below.

Table 3
Conservative Substitutions III

<u>Original Residue</u>		<u>Exemplary Substitution</u>
25		
	Ala (A)	Val, Leu, Ile
	Arg (R)	Lys, Gln, Asn
	Asn (N)	Gln, His, Lys, Arg
	Asp (D)	Glu
30	Cys (C)	Ser
	Gln (Q)	Asn
	Glu (E)	Asp

	His (H)	Asn, Gln, Lys, Arg
	Ile (I)	Leu, Val, Met, Ala, Phe,
	Leu (L)	Ile, Val, Met, Ala, Phe
	Lys (K)	Arg, Gln, Asn
5	Met (M)	Leu, Phe, Ile
	Phe (F)	Leu, Val, Ile, Ala
	Pro (P)	Gly
	Ser (S)	Thr
	Thr (T)	Ser
10	Trp (W)	Tyr
	Tyr (Y)	Trp, Phe, Thr, Ser
	Val (V)	Ile, Leu, Met, Phe, Ala

Methods Directed to the Production of Virus and Host Cells of the Invention

15 The invention provides a method of modifying production a virus that is a member of the family *Arteriviridae* and *Asfarviridae* in a cell comprising the step of directing said cell to express a CD163 polypeptide. This may include rendering a virus non-permissive cell into a virus permissive cell, or may involve rendering a cell more permissive to the virus.

20 In one embodiment, the virus that is a member of the family *Arteriviridae* or *Asfarviridae* is selected from the group consisting of LDV of mice, equine arteritis virus (EAV), simian hemorrhagic fever virus (SHFV), PRRSV of swine and ASFV of swine.

In a preferred embodiment the virus is PRRSV.

25 The invention further provides a method of preparing a culture of a virus that is a member of the family *Arteriviridae* or *Asfarviridae* comprising the steps of: providing a cell line; directing said cell line to express a CD163 polypeptide; infecting said cell line with virus; and causing said cell line to produce viral progeny.

30 In one embodiment, the virus that is a member of the family *Arteriviridae* is selected from the group consisting of LDV of mice, equine arteritis virus (EAV), simian hemorrhagic fever virus (SHFV), PRRSV of swine and ASFV of swine.

In a preferred embodiment the virus is PRRSV.

All of the above methods utilize cells and cell lines expressing a CD163 polypeptide. CD163 may be facilitated or increased by methods that involve the introduction of exogenous nucleic acid into the cell. Such a cell may comprise a polynucleotide or vector in a manner that permits expression of an encoded CD163 polypeptide.

Polynucleotides that encode CD163 may be introduced into the host cell as part of a circular plasmid, or as linear DNA comprising an isolated protein-coding region, or in a viral vector. Methods for introducing exogenous nucleic acid into the host cell well known and routinely practiced in the art include transformation, transfection, electroporation, nuclear injection, or fusion with carriers such as liposomes, micelles, ghost cells, and protoplasts. Host cell systems of the invention include invertebrate and vertebrate cells systems. Hosts may include, but are not limited to, the following: insect cells, porcine kidney (PK) cells, feline kidney (FK) cells, swine testicular (ST) cells, African green monkey kidney cells (MA-104, MARC-145, VERO, and COS cells), Chinese hamster ovary (CHO) cells, baby hamster kidney cells, human 293 cells, and murine 3T3 fibroblasts. Insect host cell culture systems may also be used for the expression of CD163 polypeptides. In another embodiment, the CD163 polypeptides are expressed using a drosophila expression system.

The choice of a suitable expression vector for expression of the CD163 polypeptides will of course depend upon the specific host cell to be used, and is within the skill of the ordinary artisan. Examples of suitable expression vectors include pSport and pcDNA3 (Invitrogen), pCMV-Script (Stratagene), and pSVL (Pharmacia Biotech). Expression vectors for use in mammalian host cells may include transcriptional and translational control sequences derived from viral genomes. Commonly used promoter sequences and modifier sequences which may be used in the present invention include, but are not limited to, those derived from human cytomegalovirus (CMV), Rous sarcoma virus (RSV), Adenovirus 2, Polyoma virus, and Simian virus 40 (SV40). Methods for the construction of mammalian expression vectors are disclosed, for example, in Okayama and Berg (*Mol. Cell. Biol.* 3:280 (1983)); Cosman *et al.* (*Mol. Immunol.* 23:935 (1986)); Cosman *et al.* (*Nature* 312:768 (1984)); EP-A-0367566; and WO 91/18982.

Because CD163 sequences are known to exist in cells from various species, the endogenous gene may be modified to permit, or increase, expression of the CD163 polypeptide. Cells can be modified (e.g., by homologous recombination) to provide increased expression by replacing, in whole or in part, the naturally occurring CD163 promoter with all or part of a heterologous promoter, so that the cells express CD163 polypeptide at higher levels. The heterologous promoter is inserted in such a manner that it is operatively linked to endogenous CD163 encoding sequences. [See, for example, PCT International Publication No. WO 94/12650, PCT International Publication No. WO 92/20808, and PCT International Publication No. WO 91/09955.] It is also contemplated that, in addition to heterologous promoter DNA, amplifiable marker DNA (e.g., *ada*, *dhfr*, and the multifunctional *cad* gene, which encodes for carbamyl phosphate synthase, aspartate transcarbamylase, and dihydroorotase) and/or intron DNA may be inserted along with the heterologous promoter DNA. If linked to the CD163 coding sequence, amplification of the marker DNA by standard selection methods results in co-amplification of the CD163 coding sequences in the cells.

CD163 expression may also be induced by chemical treatment. Phorbol esters, especially phorbol myristyl acetate (PMA), activate one or more isozymes of the ubiquitous membrane receptor, protein kinase C (PKC) and are particularly preferred means of increasing CD163 expression. Other methods of intracellular calcium mobilization are also contemplated.

Vaccine Production

The methods described above may be used to produce any virus that is a member of the family *Arteriviridae* or *Asfarviridae* for the purpose of vaccine production or diagnostics.

In one embodiment the virus that is a member of the family *Arteriviridae* is selected from the group consisting of LDV of mice, equine arteritis virus (EAV), simian hemorrhagic fever virus (SHFV), and PRRSV of swine.

In a preferred embodiment the virus is PRRSV.

Vaccine Production

The methods described above may be used to produce virus for the purpose of vaccine production or diagnostics.

Killed (inactivated) or live vaccines can be produced. Therefore, to make a live vaccine, a viral isolate, or an attenuated or mutated variant thereof, is grown in cell culture. The virus is harvested according to methods well known in the art. The virus may then be concentrated, frozen, and stored at -70°C , or freeze-dried and stored
5 at 4°C . Prior to vaccination the virus is mixed at an appropriate dosage, (which is from about 10^3 to 10^8 tissue culture infectious doses per ml ($\text{TCID}_{50}/\text{ml}$)), with a pharmaceutically acceptable carrier such as a saline solution, and optionally an adjuvant.

The vaccine produced might also comprise an inactivated or killed vaccine
10 comprising a virus grown by the methods of the invention. The inactivated vaccine is made by methods well known in the art. For example, once the virus is propagated to high titers, it would be readily apparent by those skilled in the art that the virus antigenic mass could be obtained by methods well known in the art. For example, the virus antigenic mass may be obtained by dilution, concentration, or extraction. All of
15 these methods have been employed to obtain appropriate viral antigenic mass to produce vaccines. The virus is then inactivated by treatment with formalin, betapropriolactone (BPL), binary ethyleneimine (BEI), or other methods known to those skilled in the art. The inactivated virus is then mixed with a pharmaceutically acceptable carrier such as a saline solution, and optionally an adjuvant. Examples of
20 adjuvants include, but not limited to, aluminum hydroxide, oil-in-water and water-in-oil emulsions, AMPHIGEN, saponins such as QuilA, and polypeptide adjuvants including interleukins, interferons, and other cytokines.

Inactivation by formalin is performed by mixing the viral suspension with 37% formaldehyde to a final formaldehyde concentration of 0.05%. The virus-
25 formaldehyde mixture is mixed by constant stirring for approximately 24 hours at room temperature. The inactivated virus mixture is then tested for residual live virus by assaying for growth on a suitable cell line.

Inactivation by BEI is performed by mixing the viral suspension of the present invention with 0.1 M BEI (2-bromo-ethylamine in 0.175 N NaOH) to a final BEI
30 concentration of 1 mM. The virus-BEI mixture is mixed by constant stirring for approximately 48 hours at room temperature, followed by the addition of 1.0 M sodium thiosulfate to a final concentration of 0.1 mM. Mixing is continued for an

additional two hours. The inactivated virus mixture is tested for residual live virus by assaying for growth on a suitable cell line.

Virus permissive cells that have been directed to express CD163 can also be used to quantify live virus. Two common methods, which are well known to those
5 skilled in the art, are the plaque assay and the limiting dilution assay.

CD163-expressing cell lines of the present invention can be used to grow virus for the purpose of producing viral antigen for diagnostic kits. For example lysates from infected cells (with optional purification of viral particles or extraction of selected viral proteins) may be coated on ELISA plates in order to detect and quantify
10 antibodies to the virus in swine sera.

Live or inactivated virus grown in CD163-expressing cells can be used after optional separation of the viral proteins to immunize animals in order to generate polyclonal, monospecific or monoclonal antibodies. These in turn can be used as the basis of diagnostic assays for the detection and quantification of virus in swine serum
15 and other biological samples.

Assays of the Invention

The invention provides methods for determining the propensity of an animal to be infected by a virus that is a member of the family *Arteriviridae* or *Asfarviridae* or of a cell line to support the replication of a virus that is a member of the family
20 *Arteriviridae* or *Asfarviridae*. Samples from either source are obtained and assayed for expression of CD163. The level of CD163 gene expression can be compared with levels of controls known not to support replication of the virus.

In the case of an animal, samples can be any sample comprising sample nucleic acid molecules or proteins, and obtained from any bodily tissue expressing
25 CD163, including, but not limited to, alveolar macrophages, cultured cells, biopsies, or other tissue preparations. The level of expression can be assessed at either or both the level of messenger RNA or protein produced. In a preferred embodiment the member of the virus of the family *Arteriviridae* or *Asfarviridae* is selected from the group consisting of LDV of mice, equine arteritis virus (EAV), simian hemorrhagic
30 fever virus (SHFV), PRRSV of swine, and ASFV of swine.

Nucleic Acid based Assays

Methods of determining CD163 levels may be nucleic acid based as noted above. CD163-derived nucleic acids may be in solution or on a solid support. In

some embodiments, they may be employed as array elements in microarrays alone or in combination with other array element molecules. Nucleic acid based methods generally require the isolation of DNA or RNA from the sample and subsequent hybridization or PCR amplification using specific primers derived from any known CD163 encoding sequences in the art or those specifically disclosed as SEQ ID NO: 1, 3, 5, 12, 13, 17, 18, 22, 23, 25, 26, 30, 31 33, 35, 37, 39, 41, 43, 45, and 47. DNA or RNA can be isolated from the sample according to any of a number of methods well known to those of skill in the art. For example, methods of purification of nucleic acids are described in Tijssen, P. (1993) Laboratory Techniques in Biochemistry and Molecular Biology: Hybridization With Nucleic Acid Probes, Part I. Theory and Nucleic Acid Preparation, Elsevier, New York, N.Y. In one preferred embodiment, total RNA is isolated using the TRIZOL total RNA isolation reagent (Life Technologies, Inc., Gaithersburg Md.) and mRNA is isolated using oligo d(T) column chromatography or glass beads. When sample nucleic acid molecules are amplified it is desirable to amplify the sample nucleic acid molecules and maintain the relative abundances of the original sample, including low abundance transcripts. RNA can be amplified in vitro, in situ, or in vivo (See Eberwine U.S. Pat. No. 5,514,545).

It is also advantageous to include controls within the sample to assure that amplification and labeling procedures do not change the true distribution of nucleic acid molecules in a sample. For this purpose, a sample is spiked with an amount of a control nucleic acid molecule predetermined to be detectable upon hybridization to its complementary arrayed nucleic acid molecule and the composition of nucleic acid molecules includes reference nucleic acid molecules which specifically hybridize with the control arrayed nucleic acid molecules. After hybridization and processing, the hybridization signals obtained should reflect accurately the amounts of control arrayed nucleic acid molecules added to the sample.

Prior to hybridization, it may be desirable to fragment the sample nucleic acid molecules. Fragmentation improves hybridization by minimizing secondary structure and cross-hybridization to other sample nucleic acid molecules in the sample or noncomplementary nucleic acid molecules. Fragmentation can be performed by mechanical or chemical means.

Labeling

The sample nucleic acid molecules or probes may be labeled with one or more

labeling moieties to allow for detection of hybridized arrayed/sample nucleic acid molecule complexes. The labeling moieties can include compositions that can be detected by spectroscopic, photochemical, biochemical, bioelectronic, immunochemical, electrical, optical, or chemical means. The labeling moieties include radioisotopes, such as $(^{32})\text{P}$, $(^{33})\text{P}$ or $(^{35})\text{S}$, chemiluminescent compounds, labeled binding proteins, heavy metal atoms, spectroscopic markers, such as fluorescent markers and dyes, magnetic labels, linked enzymes, mass spectrometry tags, spin labels, electron transfer donors and acceptors, and the like. Preferred fluorescent markers include Cy3 and Cy5 fluorophores (Amersham Pharmacia Biotech, Piscataway N.J.).

Hybridization

The nucleic acid molecule sequence of SEQ ID NO: 1, 3, 5, 12, 13, 17, 18, 22, 23, 25, 26, 30, 31, 33, 35, 37, 39, 41, 43, 45 and 47 or other CD163 encoding sequences in the art and fragments thereof can be used in various hybridization technologies for various purposes. Hybridization probes may be designed or derived from any mammalian CD163 sequence but may make use of those sequences disclosed in SEQ ID NO: 1, 3, 5, 12, 13, 17, 18, 22, 23, 25, 26, 30, 31, 33, 35, 37, 39, 41, 43, 45 and 47. Such probes may be made from a highly specific region or from a conserved motif, and used in protocols to quantify CD163 message, allelic variants, or related sequences. The hybridization probes of the subject invention may be DNA or RNA and may be derived from any mammalian CD163 sequence known in the art or from those sequences disclosed herein as SEQ ID NO: 1, 3, 5, 12, 13, 17, 18, 22, 23, 25, 26, 30 or from genomic sequences including promoters, enhancers, and introns of the mammalian gene. Hybridization or PCR probes may be produced using oligolabeling, nick translation, end-labeling, or PCR amplification in the presence of the labeled nucleotide. A vector containing the nucleic acid sequence may be used to produce an mRNA probe in vitro by addition of an RNA polymerase and labeled nucleic acid molecules. These procedures may be conducted using commercially available kits such as those provided by Amersham Pharmacia Biotech.

The stringency of hybridization is determined by the G+C content of the probe, salt concentration, and temperature. In particular, stringency can be increased by reducing the concentration of salt or raising the hybridization temperature. In solutions used for some membrane-based hybridizations, additions of an organic solvent such as

formamide allows the reaction to occur at a lower temperature. Hybridization can be performed at low stringency with buffers, such as 5xSSC with 1% sodium dodecyl sulfate (SDS) at 60°C., which permits the formation of a hybridization complex between nucleotide sequences that contain some mismatches. Subsequent washes are performed at higher stringency with buffers such as 0.2xSSC with 0.1% SDS at either 45°C (medium stringency) or 68°C (high stringency). At high stringency, hybridization complexes will remain stable only where the nucleic acid sequences are almost completely complementary. In some membrane-based hybridizations, preferably 35% or most preferably 50%, formamide can be added to the hybridization solution to reduce the temperature at which hybridization is performed, and background signals can be reduced by the use of other detergents such as Sarkosyl or Triton X-100 and a blocking agent such as salmon sperm DNA. Selection of components and conditions for hybridization are well known to those skilled in the art and are reviewed in Ausubel (supra) and Sambrook et al. (1989) *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Press, Plainview N.Y.

Exemplary highly stringent hybridization conditions are as follows: hybridization at 42°C in a hybridization solution comprising 50% formamide, 1% SDS, 1M NaCl, 10% Dextran sulfate, and washing twice for 30 minutes at 60°C in a wash solution comprising 0.1 X SSC and 1% SDS. It is understood in the art that conditions of equivalent stringency can be achieved through variation of temperature and buffer, or salt concentration as described Ausubel, et al. (Eds.), *Protocols in Molecular Biology*, John Wiley & Sons (1994), pp. 6.0.3 to 6.4.10. Modifications in hybridization conditions can be empirically determined or precisely calculated based on the length and the percentage of guanosine/cytosine (GC) base pairing of the probe. The hybridization conditions can be calculated as described in Sambrook, et al., (Eds.), *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press: Cold Spring Harbor, New York (1989), pp. 9.47 to 9.51.

Hybridization specificity can be evaluated by comparing the hybridization of specificity-control nucleic acid molecules to specificity-control sample nucleic acid molecules that are added to a sample in a known amount. The specificity-control arrayed nucleic acid molecules may have one or more sequence mismatches compared with the corresponding arrayed nucleic acid molecules. In this manner, it is possible to determine whether only complementary arrayed nucleic acid molecules are

hybridizing to the sample nucleic acid molecules or whether mismatched hybrid duplexes are forming is determined.

Hybridization reactions can be performed in absolute or differential hybridization formats. In the absolute hybridization format, nucleic acid molecules from one sample are hybridized to the molecules in a microarray format and signals detected after hybridization complex formation correlate to nucleic acid molecule levels in a sample. In the differential hybridization format, the differential expression of a set of genes in two biological samples is analyzed. For differential hybridization, nucleic acid molecules from both biological samples are prepared and labeled with different labeling moieties. A mixture of the two labeled nucleic acid molecules is added to a microarray. The microarray is then examined under conditions in which the emissions from the two different labels are individually detectable. Molecules in the microarray that are hybridized to substantially equal numbers of nucleic acid molecules derived from both biological samples give a distinct combined fluorescence (Shalon et al.; PCT publication WO95/35505). In a preferred embodiment, the labels are fluorescent markers with distinguishable emission spectra, such as Cy3 and Cy5 fluorophores.

After hybridization, the microarray is washed to remove nonhybridized nucleic acid molecules and complex formation between the hybridizable array elements and the nucleic acid molecules is detected. Methods for detecting complex formation are well known to those skilled in the art. In a preferred embodiment, the nucleic acid molecules are labeled with a fluorescent label and measurement of levels and patterns of fluorescence indicative of complex formation is accomplished by fluorescence microscopy, preferably confocal fluorescence microscopy.

In a differential hybridization experiment, nucleic acid molecules from two or more different biological samples are labeled with two or more different fluorescent labels with different emission wavelengths. Fluorescent signals are detected separately with different photomultipliers set to detect specific wavelengths. The relative abundances/ expression levels of the nucleic acid molecules in two or more samples are obtained.

Typically, microarray fluorescence intensities can be normalized to take into account variations in hybridization intensities when more than one microarray is used under similar test conditions. In a preferred embodiment, individual arrayed-sample

nucleic acid molecule complex hybridization intensities are normalized using the intensities derived from internal normalization controls contained on each microarray.

Polypeptide Based Assays

The present invention provides methods and reagents for detecting and
5 quantifying CD163 polypeptides. These methods include analytical biochemical methods such as electrophoresis, mass spectroscopy, chromatographic methods and the like, or various immunological methods such as radioimmunoassay (RIA), enzyme-linked immunosorbent assays (ELISAs), immunofluorescent assays, western blotting, affinity capture mass spectrometry, biological activity, and others described
10 below and apparent to those of skill in the art upon review of this disclosure.

Immunoassays

The present invention also provides methods for detection of CD163 polypeptides employing one or more anti- CD163 antibody reagents (i.e., immunoassays). As used herein, an immunoassay is an assay that utilizes an antibody (as broadly defined herein
15 and specifically includes fragments, chimeras and other binding agents) that specifically binds a CD163 polypeptide or epitope.

A number of well-established immunological binding assay formats suitable for the practice of the invention are known (see, e.g., U.S. Pat. Nos. 4,366,241; 4,376,110; 4,517,288; and 4,837,168). See, e.g., Methods in Cell Biology Volume 37:
20 Antibodies in Cell Biology, Asai, ed. Academic Press, Inc. New York (1993); Basic and Clinical Immunology 7th Edition, Stites & Terr, eds. (1991); Harlow and Lane, supra [e.g., Chapter 14], and Ausubel et al., supra, [e.g., Chapter 11]. Typically, immunological binding assays (or immunoassays) utilize a "capture agent" to specifically bind to and, often, immobilize the analyte to a solid phase. In one
25 embodiment, the capture agent is a moiety that specifically binds to a CD163 polypeptide or subsequence, such as an anti- CD163 antibody.

Usually the CD163 gene product being assayed is detected directly or indirectly using a detectable label. The particular label or detectable group used in the assay is usually not a critical aspect of the invention, so long as it does not
30 significantly interfere with the specific binding of the antibody or antibodies used in the assay. The label may be covalently attached to the capture agent (e.g., an anti-CD163 antibody), or may be attached to a third moiety, such as another antibody, that specifically binds to the CD163 polypeptide.

The present invention provides methods and reagents for competitive and noncompetitive immunoassays for detecting CD163 polypeptides. Noncompetitive immunoassays are assays in which the amount of captured analyte (in this case CD163) is directly measured. One such assay is a two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on the CD163 polypeptide. See, e.g., Maddox et al., 1983, J. Exp. Med., 158:1211 for background information. In one "sandwich" assay, the capture agent (e.g., an anti-CD163 antibody) is bound directly to a solid substrate where it is immobilized. These immobilized antibodies then capture any CD163 polypeptide present in the test sample. The CD163 polypeptide thus immobilized can then be labeled, i.e., by binding to a second anti-CD163 antibody bearing a label. Alternatively, the second CD163 antibody may lack a label, but be bound by a labeled third antibody specific to antibodies of the species from which the second antibody is derived. The second antibody alternatively can be modified with a detectable moiety, such as biotin, to which a third labeled molecule can specifically bind, such as enzyme-labeled streptavidin.

In competitive assays, the amount of CD163 polypeptide present in the sample is measured indirectly by measuring the amount of an added (exogenous) CD163 polypeptide displaced (or competed away) from a capture agent (e.g., CD163 antibody) by the CD163 polypeptide present in the sample. A hapten inhibition assay is another example of a competitive assay. In this assay CD163 polypeptide is immobilized on a solid substrate. A known amount of CD163 antibody is added to the sample, and the sample is then contacted with the immobilized CD163 polypeptide. In this case, the amount of anti-CD163 antibody bound to the immobilized CD163 polypeptide is inversely proportional to the amount of CD163 polypeptide present in the sample. The amount of immobilized antibody may be detected by detecting either the immobilized fraction of antibody or the fraction of the antibody that remains in solution. In this aspect, detection may be direct, where the antibody is labeled, or indirect where the label is bound to a molecule that specifically binds to the antibody as described above.

Other Antibody-based Assay Formats

The invention also provides reagents and methods for detecting and quantifying the presence of CD163 polypeptide in the sample by using an immunoblot (Western blot)

format. Another immunoassay is the so-called "lateral flow chromatography." In a non-competitive version of lateral flow chromatography, a sample moves across a substrate by, e.g., capillary action, and encounters a mobile-labeled antibody that binds the analyte forming a conjugate. The conjugate then moves across the substrate and encounters an immobilized second antibody that binds the analyte. Thus, immobilized analyte is detected by detecting the labeled antibody. In a competitive version of lateral flow chromatography a labeled version of the analyte moves across the carrier and competes with unlabeled analyte for binding with the immobilized antibody. The greater the amount of the analyte in the sample, the less the binding by labeled analyte and, therefore, the weaker the signal. See, e.g., May et al., U.S. Pat. No. 5,622,871 and Rosenstein, U.S. Pat. No. 5,591,645.

Depending upon the assay, various components, including the antigen, target antibody, or anti-cathepsin S antibody, may be bound to a solid surface or support (i.e., a substrate, membrane, or filter paper). Many methods for immobilizing biomolecules to a variety of solid surfaces are known in the art. For instance, the solid surface may be a membrane (e.g., nitrocellulose), a microtiter dish (e.g., PVC, polypropylene, or polystyrene), a test tube (glass or plastic), a dipstick (e.g. glass, PVC, polypropylene, polystyrene, latex, and the like), a microcentrifuge tube, or a glass or plastic bead. The desired component may be covalently bound or noncovalently attached through nonspecific bonding.

A wide variety of organic and inorganic polymers, both natural and synthetic may be employed as the material for the solid surface. Illustrative polymers include polyethylene, polypropylene, poly(4-methylbutene), polystyrene, polymethacrylate, poly(ethylene terephthalate), rayon, nylon, poly(vinylbutyrate), polyvinylidene difluoride (PVDF), silicones, polyformaldehyde, cellulose, cellulose acetate, nitrocellulose, and the like. Other materials which may be employed, include paper, glasses, ceramics, metals, metalloids, semiconductive materials, cements or the like. In addition, substances that form gels, such as proteins (e. g., gelatins), lipopolysaccharides, silicates, agarose and polyacrylamides can be used. Polymers that form several aqueous phases, such as dextrans, polyalkylene glycols or surfactants, such as phospholipids, long chain (12-24 carbon atoms) alkyl ammonium salts and the like are also suitable. Where the solid surface is porous, various pore sizes may be employed depending upon the nature of the system.

Mass Spectrometry

The mass of a molecule frequently can be used as an identifier of the molecule.

Therefore, methods of mass spectrometry can be used to identify a protein analyte.

Mass spectrometers can measure mass by determining the time required for an ionized
5 analyte to travel down a flight tube and to be detected by an ion detector. One method
of mass spectrometry for proteins is matrix-assisted laser desorption ionization mass
spectrometry ("MALDI"). In MALDI the analyte is mixed with an energy absorbing
matrix material that absorbs energy of the wavelength of a laser and placed on the
surface of a probe. Upon striking the matrix with the laser, the analyte is desorbed
10 from the probe surface, ionized, and detected by the ion detector. See, for example,
Hillenkamp et al., U.S. Pat. No. 5,118,937.

Other methods of mass spectrometry for proteins are described in Hutchens
and Yip, U.S. Pat. No. 5,719,060. In one such method referred to as Surfaces
Enhanced for Affinity Capture ("SEAC") a solid phase affinity reagent that binds the
15 analyte specifically or non-specifically, such as an antibody or a metal ion, is used to
separate the analyte from other materials in a sample. Then the captured analyte is
desorbed from the solid phase by, e.g., laser energy, ionized, and detected by the
detector.

Nucleic Acids of the Invention

20 The examples disclose our discovery of several novel CD163 polynucleotides.
The invention includes these novel CD163 polynucleotides. The present invention
provides several isolated novel polynucleotides (e.g., DNA sequences and RNA
transcripts, both sense and complementary antisense strands, both single and double-
stranded, including splice variants thereof, which encode novel CD163 polypeptides.
25 We report herein isolated novel polynucleotides which encode porcine, murine,
human, canine, and african green monkey CD163 polypeptides and which comprise
the sequences set forth in SEQ ID NO: 1, 5, 12 13, 22, 23, 25, 26, 30 31, 33, 35, 37,
39, 41, 43, 45, and 47.

It should be recognized that by disclosing SEQ ID NOs: 1, 5, 12 13, 22, 23, 25,
30 26, 30 31, 33, 35, 37, 39, 41, 43, 45, and 47 it provides one skilled in the art a
multitude of methods of obtaining these sequences. By way of example, it would be
possible to generate probes from the sequence disclosed in SEQ ID NOs: 1, 5, 12 13,
22, 23, 25, 26, 30 31, 33, 35, 37, 39, 41, 43, 45, and 47 and screen porcine, murine,

human, canine, and african green monkey cDNA or genomic libraries and thereby obtain the entire SEQ ID NO: 1, 3, 5, 12, 13, 17, 18, 22, 23, 25, 26, 30, 31, 33, 35, 37, 39, 41, 43, 45 and 47, or its genomic equivalent. Sambrook, *et al.*, (Eds.), *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press: Cold Spring Harbor, New York (1989). Also by way of example, one skilled in the art would immediately recognize that given the sequence disclosed in SEQ ID NO: 5, 12 13, 22, 23, 25, 26, 30 31, 33, 35, 37, 39, 41, 43, 45, and 47 it is then possible to generate the appropriate primers for PCR amplification to obtain the entire sequence represented by these sequences. (See e.g., PCR Technology, H. A. Erlich, ed., Stockton Press, New York, 1989; PCR Protocols: A Guide to Methods and Applications, M. A. Innis, David H. Gelfand, John J. Sninsky, and Thomas J. White, eds., Academic Press, Inc., New York, 1990.)

DNA polynucleotides of the invention include cDNA, and DNA that has been chemically synthesized in whole or in part and is also intended to include allelic variants thereof. Allelic variants are modified forms of a wild type gene sequence, the modification resulting from recombination during chromosomal segregation, or exposure to conditions which give rise to genetic mutation. Allelic variants, like wild type genes, are naturally occurring sequences (as opposed to non-naturally occurring variants which arise from in vitro manipulation).

DNA sequences encoding the novel CD163 polypeptides are set forth in SEQ ID NOs: 5, 12 13, 22, 23, 25, 26, 30 31, 33, 35, 37, 39, 41, 43, 45, and 47. The worker of skill in the art will readily appreciate that the DNA of the invention comprises a double stranded molecule, for example the molecule having the sequence set forth in SEQ ID NO: 5, 12 13, 22, 23, 25, 26, 30 31, 33, 35, 37, 39, 41, 43, 45, and 47 along with the complementary molecule (the "non-coding strand" or "complement") having a sequence deducible from the sequence of SEQ ID NOs: 5, 12 13, 22, 23, 25, 26, 30 31, 33, 35, 37, 39, 41, 43, 45, and 47 according to Watson-Crick base pairing rules for DNA. Also contemplated by the invention are other polynucleotides encoding for the porcine, murine and african green monkey CD163 polypeptides of SEQ ID NO: 2, 14, 24, 27 and 32, 34, 36, 38, 40, 42, 44, 46, 48 which differ in sequence from the polynucleotide of SEQ ID NOs: 1, 3, 5, 12, 13, 17, 18, 22, 23, 25, 26, 30, 31, 33, 35, 37, 39, 41, 43, 45 and 47 by virtue of the well-known degeneracy of the universal genetic code, as is well known in the art. The

present invention, therefore, contemplates those other DNA and RNA molecules that, on expression, encode the polypeptides of SEQ ID NO: 2, 14, 24, 27 and 32. Having identified the amino acid residue sequence encoded the porcine CD163 polypeptide, and with the knowledge of all triplet codons for each particular amino acid residue, it is possible to describe all such encoding RNA and DNA sequences. DNA and RNA molecules other than those specifically disclosed herein characterized simply by a change in a codon for a particular amino acid, are, therefore, within the scope of this invention.

A table of amino acids and their representative abbreviations, symbols and codons is set forth below in the following Table 4.

Table 4

Amino acid	Abbrev.	Symbol	Codon(s)					
Alanine	Ala	A	GCA	GCC	GCG	GCU		
Cysteine	Cys	C	UGC	UGU				
Aspartic acid	Asp	D	GAC	GAU				
Glutamic acid	Glu	E	GAA	GAG				
Phenylalanine	Phe	F	UUC	UUU				
Glycine	Gly	G	GGA	GGC	GGG	GGU		
Histidine	His	H	CAC	CAU				
Isoleucine	Ile	I	AUA	AUC	AUU			
Lysine	Lys	K	AAA	AAG				
Leucine	Leu	L	UUA	UUG	CUA	CUC	CUG	CUU
Methionine	Met	M	AUG					
Asparagine	Asn	N	AAC	AAU				
Proline	Pro	P	CCA	CCC	CCG	CCU		
Glutamine	Gln	Q	CAA	CAG				
Arginine	Arg	R	AGA	AGG	CGA	CGC	CGG	CGU
Serine	Ser	S	AGC	AGU	UCA	UCC	UCG	UCU
Threonine	Thr	T	ACA	ACC	ACG	ACU		
Valine	Val	V	GUA	GUC	GUG	GUU		
Tryptophan	Trp	W	UGG					
Tyrosine	Tyr	Y	UAC	UAU				

As is well known in the art, codons constitute triplet sequences of nucleotides in mRNA and their corresponding cDNA molecules. Codons are characterized by the base uracil (U) when present in an mRNA molecule but are characterized by the base thymidine (T) when present in DNA. A simple change in a codon for the same amino acid residue within a polynucleotide will not change the sequence or structure of the encoded polypeptide. It is apparent that when a phrase stating that a particular 3 nucleotide sequence "encode(s)" any particular amino acid, the ordinarily skilled artisan would recognize that the table above provides a means of identifying the particular nucleotides at issue. By way of example, if a particular three-nucleotide

sequence encodes theonine the table above discloses that the possible triplet sequences are ACA, ACG, ACC and ACU (ACT if in DNA).

The invention includes therefore, an isolated polynucleotide comprising:

- (a) a susCD163v1 polynucleotide sequence set forth in SEQ ID NOs: 1, and 5
- 5 (b) a polynucleotide that encodes a polypeptide that has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 93%, 94%, 95%, 96% 97% 98%, or 99%, identity and/or similarity to a polypeptide set forth in SEQ ID NO: 2
- (c) a polynucleotide encoding a polypeptide of SEQ ID: 2,
- 10 (d) a polynucleotide which is the complement of any of (a), (b) or (c).

The invention also includes an isolated polynucleotide comprising:

- (a) a susCD163v2 polynucleotide sequence set forth in SEQ ID NOs: 12 or 13
- (b) a polynucleotide that encodes a polypeptide that has at least 99%, identity and/or similarity to a polypeptide set forth in SEQ ID NO: 14
- 15 (c) a polynucleotide encoding a polypeptide of SEQ ID: 14,
- (d) a polynucleotide which is the complement of any of (a), (b) or (c).

The invention also includes an isolated polynucleotide comprising:

- (a) a murine CD63v2 polynucleotide sequence set forth in SEQ ID NOs: 22 or 23
- (b) a polynucleotide encoding a polypeptide of SEQ ID: 24,
- 20 (c) a polynucleotide which is the complement of any of (a) or (b).

The invention also includes an isolated polynucleotide comprising:

- (a) a murine CD163v3 polynucleotide sequence set forth in SEQ ID NOs: 25 or 26
- (b) a polynucleotide that encodes a polypeptide that has at least 96% 97% 98%, or 99% identity and/or similarity to a polypeptide set forth in SEQ ID NO: 27
- 25 (c) a polynucleotide encoding a polypeptide of SEQ ID: 27,
- (d) a polynucleotide which is the complement of any of (a), (b) or (c).

The invention also includes an isolated polynucleotide comprising:

- (a) an african green monkey CD163v2 polynucleotide sequence set forth in SEQ ID NOs: 30 or 31
- 30 (b) a polynucleotide that encodes a polypeptide that has at least 98% or 99% identity and/or similarity to a polypeptide set forth in SEQ ID NO: 32
- (c) a polynucleotide encoding a polypeptide of SEQ ID: 32,
- (d) a polynucleotide which is the complement of any of (a), (b) or (c).

The invention also includes an isolated polynucleotide comprising:

- (a) a polynucleotide sequence set forth in SEQ ID NO: 33
- (b) a polynucleotide that encodes a polypeptide that has at least 95% 96%, 97%, 98% or 99% identity and/or similarity to a polypeptide set forth in SEQ ID NO: 34
- 5 (c) a polynucleotide encoding a polypeptide of SEQ ID: 34,
- (d) a polynucleotide which is the complement of any of (a), (b) or (c).

The invention also includes an isolated polynucleotide comprising:

- (a) a polynucleotide sequence set forth in SEQ ID NO: 35
- (b) a polynucleotide that encodes a polypeptide that has at least 95% 96%, 97%, 98% or
- 10 99% identity and/or similarity to a polypeptide set forth in SEQ ID NO: 36
- (c) a polynucleotide encoding a polypeptide of SEQ ID: 36,
- (d) a polynucleotide which is the complement of any of (a), (b) or (c).

The invention also includes an isolated polynucleotide comprising:

- (a) a polynucleotide sequence set forth in SEQ ID NO: 37
- 15 (b) a polynucleotide that encodes a polypeptide that has at least 95% 96%, 97%, 98% or 99% identity and/or similarity to a polypeptide set forth in SEQ ID NO: 38
- (c) a polynucleotide encoding a polypeptide of SEQ ID: 38,
- (d) a polynucleotide which is the complement of any of (a), (b) or (c).

The invention also includes an isolated polynucleotide comprising:

- 20 (a) a polynucleotide sequence set forth in SEQ ID NO: 39
- (b) a polynucleotide that encodes a polypeptide that has at least 95% 96%, 97%, 98% or 99% identity and/or similarity to a polypeptide set forth in SEQ ID NO: 40
- (c) a polynucleotide encoding a polypeptide of SEQ ID: 40,
- (d) a polynucleotide which is the complement of any of (a), (b) or (c).

25 The invention also includes an isolated polynucleotide comprising:

- (a) a polynucleotide sequence set forth in SEQ ID NO: 41
- (b) a polynucleotide that encodes a polypeptide that has at least 95% 96%, 97%, 98% or 99% identity and/or similarity to a polypeptide set forth in SEQ ID NO: 42
- (c) a polynucleotide encoding a polypeptide of SEQ ID: 42,
- 30 (d) a polynucleotide which is the complement of any of (a), (b) or (c).

The invention also includes an isolated polynucleotide comprising:

- (a) a polynucleotide sequence set forth in SEQ ID NO: 43

(b) a polynucleotide that encodes a polypeptide that has at least 95% 96%, 97%, 98% or 99% identity and/or similarity to a polypeptide set forth in SEQ ID NO: 44

(c) a polynucleotide encoding a polypeptide of SEQ ID: 44,

(d) a polynucleotide which is the complement of any of (a), (b) or (c).

5 The invention also includes an isolated polynucleotide comprising:

(a) a polynucleotide sequence set forth in SEQ ID NO: 45

(b) a polynucleotide that encodes a polypeptide that has at least 90%, 91%, 92%, 93%, 94%, 95% 96%, 97%, 98% or 99% identity and/or similarity to a polypeptide set forth in SEQ ID NO: 46

10 (c) a polynucleotide encoding a polypeptide of SEQ ID: 46,

(d) a polynucleotide which is the complement of any of (a), (b) or (c).

The invention also includes an isolated polynucleotide comprising:

(a) a polynucleotide sequence set forth in SEQ ID NO: 47

15 (b) a polynucleotide that encodes a polypeptide that has at least 90%, 91%, 92%, 93%, 94%, 95% 96%, 97%, 98% or 99% identity and/or similarity to a polypeptide set forth in SEQ ID NO: 48

(c) a polynucleotide encoding a polypeptide of SEQ ID: 49,

(d) a polynucleotide which is the complement of any of (a), (b) or (c).

20 The polynucleotide sequence information provided by the invention makes possible large-scale expression of the encoded polypeptide by techniques well known and routinely practiced in the art. Polynucleotides of the invention also permit identification and isolation of polynucleotides encoding related porcine CD163v1 polypeptides, such as human allelic variants and species homologs, by well-known techniques including Southern and/or Northern hybridization, and the polymerase
25 chain reaction (PCR).

Knowledge of the sequence of a any of the CD163 sequences disclosed herein also makes possible through use of Southern hybridization or polymerase chain reaction (PCR), the identification of genomic DNA sequences encoding CD163 regulatory sequences, such as promoters, operators, enhancers, repressors, and the
30 like.

As noted in the section above entitled "Assays of the Invention" polynucleotides of the invention are also useful in hybridization assays to detect the capacity of cells to express CD163, or to measure levels of CD163 expression.

Polynucleotides of the invention may also be the basis for diagnostic methods useful for determining the susceptibility of an animal to virus infection as described above.

The disclosure herein of the full-length polynucleotides encoding a CD163 polypeptide makes readily available to the worker of ordinary skill in the art fragments of the full length polynucleotide. The invention therefore provides unique fragments of the CD163 encoding polynucleotides comprising at least 15 through the length of the full-length sequence (including each and every integer value between) consecutive nucleotides of a polynucleotide encoding a CD163 disclosed herein. Because polynucleotides of the invention (including fragments) comprise sequences unique to the particular CD163-encoding polynucleotide sequence, they therefore would hybridize under highly stringent or moderately stringent conditions only (i.e., "specifically") to polynucleotides encoding the various CD163 polypeptides. Sequences unique to polynucleotides of the invention are recognizable through sequence comparison to other known polynucleotides, and can be identified through use of alignment programs routinely utilized in the art, e.g., those made available in public sequence databases. Such sequences also are recognizable from Southern hybridization analyses to determine the number of fragments of genomic DNA to which a polynucleotide will hybridize. Polynucleotides of the invention can be labeled in a manner that permits their detection, including radioactive, fluorescent, and enzymatic labeling.

One or more unique fragment polynucleotides (or other CD163 polynucleotides as discussed above) can be included in kits that are used to detect the presence of a polynucleotide encoding for CD163, or used to detect variations in a polynucleotide sequence encoding for CD163. Also made available by the invention are anti-sense polynucleotides that recognize and hybridize to polynucleotides encoding CD163. Full length and fragment anti-sense polynucleotides are provided. Fragment anti-sense molecules of the invention include (i) those that specifically recognize and hybridize to the CD163 variants disclosed herein (as determined by sequence comparison of DNA encoding CD163s to DNA encoding other known molecules). Identification of sequences unique to the novel CD163-encoding polynucleotides can be deduced through the use of any publicly available sequence database, and/or through the use of commercially available sequence comparison programs. The uniqueness of selected sequences in an entire genome can be further

verified by hybridization analyses. After identification of the desired sequences, isolation through restriction digestion or amplification using any of the various polymerase chain reaction techniques well known in the art can be performed. Anti-sense polynucleotides are particularly relevant to regulating expression of CD163 by those cells expressing CD163 mRNA.

Antisense nucleic acids (preferably 10 to 20 base pair oligonucleotides) capable of specifically binding to CD163 expression control sequences or CD163 RNA are introduced into cells (e.g., by a viral vector or colloidal dispersion system such as a liposome). The antisense nucleic acid binds to the porcine CD163 target nucleotide sequence in the cell and prevents transcription or translation of the target sequence. Phosphorothioate and methylphosphonate antisense oligonucleotides are specifically contemplated for therapeutic use by the invention. The antisense oligonucleotides may be further modified by poly-L-lysine, transferrin polylysine, or cholesterol moieties at their 5' end. Suppression of porcine CD163 expression at either the transcriptional or translational level is useful to generate cellular or animal models for diseases characterized by aberrant porcine CD163 expression or as a therapeutic modality.

As noted above in more detail, the nucleic acids of the invention include vectors comprising a polynucleotide of the invention. Such vectors are useful, e.g., for amplifying the polynucleotides in host cells to create useful quantities thereof. In other embodiments, the vector is an expression vector wherein the polynucleotide of the invention is operatively linked to a polynucleotide comprising an expression control sequence. Such vectors are useful for recombinant production of polypeptides of the invention.

Also as noted above the invention provides host cells that are transformed or transfected (stably or transiently) with polynucleotides of the invention or vectors of the invention. As stated above, such host cells are useful for the production of virus and the production of vaccines.

The invention also provides isolated CD163 polypeptides encoded by a novel polynucleotide of the invention.

Polypeptides of the Invention

The examples disclose our discovery of several novel CD163 polypeptides. The invention includes these novel CD163 polypeptide which are set forth in SEQ ID NOs: 2, 14, 19, 24, 27, 32, 34, 36, 38, 40, 42, 44, 46, and 48.

5 The invention includes therefore, an isolated polynucleotide comprising a susCD163v1 polypeptide with the sequence set forth in SEQ ID NO: 2.

 The invention also includes a polypeptide that has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 93%, 94%, 95%, 96% 97% 98%, or 99%,
10 identity and/or similarity to a polypeptide set forth in SEQ ID NO: 2.

 The invention includes therefore, an isolated polynucleotide comprising a susCD163v2 polypeptide with the sequence set forth in SEQ ID NO: 14.

 The invention also includes a polypeptide that has at least 99%, identity and or similarity to a sus CD163v2 polypeptide set forth in SEQ ID NO: 14.

15 The invention also includes a murine CD163v2 polypeptide having the sequence set forth in SEQ ID: 24.

 The invention also includes a murine CD163v3 polypeptide having the sequence set forth in SEQ ID: 27.

 The invention also includes at least a polypeptide having 96% 97% 98%, or
20 99%, identity and/or similarity to a polypeptide set forth in SEQ ID NO: 27.

 The invention also includes a polypeptide having the sequence set forth in SEQ ID: 32.

 The invention also includes a polypeptide that has at least 98% or 99%, identity and/or similarity to a polypeptide set forth in SEQ ID NO: 32.

25 The invention also includes a polypeptide having the sequence set forth in SEQ ID: 34.

 The invention also includes a polypeptide that has at least 95%, 96%, 97%, 98%, 99% identity and/or similarity to a polypeptide set forth in SEQ ID NO: 34.

 The invention also includes a polypeptide having the sequence set forth in SEQ ID:
30 36.

 The invention also includes a polypeptide that has at least 95%, 96%, 97%, 98%, 99% identity and/or similarity to a polypeptide set forth in SEQ ID NO: 36.

The invention also includes a polypeptide having the sequence set forth in SEQ ID: 38.

The invention also includes a polypeptide that has at least 95%, 96%, 97%, 98%, 99% identity and/or similarity to a polypeptide set forth in SEQ ID NO: 39.

5 The invention also includes a polypeptide having the sequence set forth in SEQ ID: 40.

The invention also includes a polypeptide that has at least 95%, 96%, 97%, 98%, 99% identity and/or similarity to a polypeptide set forth in SEQ ID NO: 40.

10 The invention also includes a polypeptide having the sequence set forth in SEQ ID: 42.

The invention also includes a polypeptide that has at least 90%, 91%, 92%, 93%, 94% 95%, 96%, 97%, 98%, 99% identity and/or similarity to a polypeptide set forth in SEQ ID NO: 42.

15 The invention also includes a polypeptide having the sequence set forth in SEQ ID: 44.

The invention also includes a polypeptide that has at least 90%, 91%, 92%, 93%, 94% 95%, 96%, 97%, 98%, 99% identity and/or similarity to a polypeptide set forth in SEQ ID NO: 44.

20 The invention also includes a polypeptide having the sequence set forth in SEQ ID: 46.

The invention also includes a polypeptide that has at least 90%, 91%, 92%, 93%, 94% 95%, 96%, 97%, 98%, 99% identity and/or similarity to a polypeptide set forth in SEQ ID NO: 46.

25 The invention also includes a polypeptide having the sequence set forth in SEQ ID: 48.

The invention also includes a polypeptide that has at least 90%, 91%, 92%, 93%, 94% 95%, 96%, 97%, 98%, 99% identity and/or similarity to a polypeptide set forth in SEQ ID NO: 48.

30 Polypeptides of the invention may be isolated from natural cell sources or may be chemically synthesized, but are preferably produced by recombinant procedures involving host cells of the invention. Use of mammalian host cells is expected to provide for such post-translational modifications (e.g., glycosylation, truncation,

lipidation, and phosphorylation) as may be needed to confer optimal biological activity on recombinant expression products of the invention. Glycosylated and non-glycosylated forms of the novel CD163 polypeptides are embraced.

Overexpression in eukaryotic and prokaryotic hosts as described above
5 facilitates the isolation of CD163 polypeptides. The invention therefore includes isolated CD163 polypeptides as set out in SEQ ID NOs: 2, 14, 19, 24, 27 32, 34, 36, 38, 40, 42, 44, 46, 48 and variants and conservative amino acid substitutions therein including labeled and tagged polypeptides.

The invention includes novel CD163 polypeptides that are "labeled". The
10 term "labeled" is used herein to refer to the conjugating or covalent bonding of any suitable detectable group, including enzymes (e.g., horseradish peroxidase, beta - glucuronidase, alkaline phosphatase, and beta-D-galactosidase), fluorescent labels (e.g., fluorescein, luciferase), and radiolabels (e.g., ^{14}C , ^{125}I , ^3H , ^{32}P , and ^{35}S) to the compound being labeled. Techniques for labeling various compounds, including
15 proteins, peptides, and antibodies, are well known. See, e.g., Morrison, Methods in Enzymology 32b, 103 (1974); Syvanen et al., J. Biol. Chem. 284, 3762 (1973); Bolton and Hunter, Biochem. J. 133, 529 (1973). The termed labeled may also encompass a polypeptide which has covalently attached an amino acid tag as discussed below.

20 In addition, the novel CD163 polypeptides of the invention may be indirectly labeled. This involves the covalent addition of a moiety to the polypeptide and subsequent coupling of the added moiety to a label or labeled compound that exhibits specific binding to the added moiety. Possibilities for indirect labeling include biotinylation of the peptide followed by binding to avidin coupled to one of the above
25 label groups. Another example would be incubating a radiolabeled antibody specific for a histidine tag with a CD163s polypeptide comprising a polyhistidine tag. The net effect is to bind the radioactive antibody to the polypeptide because of the considerable affinity of the antibody for the tag.

The invention also embraces variants (or analogs) of the novel CD163 protein.
30 In one example, insertion variants are provided wherein one or more amino acid residues supplement a novel CD163 amino acid sequence. Insertions may be located at either or both termini of the protein, or may be positioned within internal regions of the novel CD163 protein amino acid sequence. Insertional variants with additional

residues at either or both termini can include for example, fusion proteins and proteins including amino acid tags or labels. Insertion variants include novel CD163 polypeptides wherein one or more amino acid residues are added to a CD163 acid sequence, or to a biologically active fragment thereof.

5 Insertional variants therefore can also include fusion proteins wherein the amino and/or carboxy termini of the novel CD163 polypeptide is fused to another polypeptide. Various tag polypeptides and their respective antibodies are well known in the art. Examples include poly-histidine (poly-his) or poly-histidine-glycine (poly-his-gly) tags; the influenza HA tag polypeptide and its antibody
10 12CA5 [Field et al., Mol. Cell. Biol., 8:2159-2165 (1988)]; the c-myc tag and the 8F9, 3C7, 6E10, G4, B7 and 9E10 antibodies thereto [Evan et al., Molecular and Cellular Biology, 5:3610-3616 (1985)], and the Herpes Simplex virus glycoprotein D (gD) tag and its antibody [Paborsky et al., Protein Engineering, 3(6):547-553 (1990)]. Other tag polypeptides include the Flag -peptide [Hopp et al.,
15 BioTechnology, 6:1204-1210 (1988)]; the KT3 epitope peptide [Martin et al., Science, 255:192-194 (1992)]; an alpha -tubulin epitope peptide [Skinner et al., J. Biol. Chem., 266:15163-15166 (1991)]; and the T7 gene 10 protein peptide tag [Lutz-Freyermuth et al., Proc. Natl. Acad. Sci. USA, 87:6393-6397(1990)]. In addition, the CD163 polypeptide can be tagged with enzymatic proteins such as
20 peroxidase and alkaline phosphatase.

 In another aspect, the invention provides deletion variants wherein one or more amino acid residues in a novel CD163 polypeptide is removed. Deletions can be effected at one or both termini of the novel CD163 polypeptide, or with removal of one or more residues within the novel CD163 amino acid sequence. Deletion
25 variants, therefore, include all fragments of the novel CD163 polypeptide.

 CD163 polypeptides contain a transmembrane or membrane anchor region. It should be recognized that such transmembrane domains are useful when expressed in the context of a heterologous protein to aid in the targeting of the heterologous protein to membranes. It should also be recognized that it may be advantageous to delete
30 some transmembrane domains to enhance the purification or solubility of the protein. Transmembrane deleted variants of CD163 and polynucleotides encoding them are of potential value as antiviral therapeutics. Such variants are specifically disclosed here as SEQ ID NOs: 37-40.

The present invention also includes variants of the aforementioned polypeptides, that is, polypeptides that vary from the reference sequence by conservative amino acid substitutions,

Exemplary conservative substitutions are set out in Tables 1, 2 and 3 in the
5 section above entitled "Definitions".

In those situations where it is preferable to partially or completely isolate the novel CD163 polypeptides, purification can be accomplished using standard methods well known to the skilled artisan. Such methods include, without limitation, separation by electrophoresis followed by electroelution, various types of
10 chromatography (immunoaffinity, molecular sieve, and/or ion exchange), and/or high pressure liquid chromatography. In some cases, it may be preferable to use more than one of these methods for complete purification.

Purification of novel CD163 polypeptides can be accomplished using a variety of techniques. If the polypeptide has been synthesized such that it contains a
15 tag such as Hexahistidine (CD163 /hexaHis) or other small peptide such as FLAG (Eastman Kodak Co., New Haven, Conn.) or myc (Invitrogen, Carlsbad, Calif.) at either its carboxyl or amino terminus, it may essentially be purified in a one-step process by passing the solution through an affinity column where the column matrix has a high affinity for the tag or for the polypeptide directly (i.e., a monoclonal
20 antibody specifically recognizing CD163). For example, polyhistidine binds with great affinity and specificity to nickel, thus an affinity column of nickel (such as the Qiagen Registered TM nickel columns) can be used for purification of CD163 /polyHis. (See for example, Ausubel et al., eds., Current Protocols in Molecular Biology, Section 10.11.8, John Wiley & Sons, New York [1993]).

25 Even if the novel CD163 polypeptide is prepared without a label or tag to facilitate purification, the novel CD163 of the invention may be purified by immunoaffinity chromatography. To accomplish this, antibodies specific for CD163 polypeptides must be prepared by means well known in the art.

Antibodies generated against the novel CD163 polypeptides of the invention
30 can be obtained by administering the polypeptides or epitope-bearing fragments, analogues, or cells to an animal, preferably a non-human, using routine protocols. For preparation of monoclonal antibodies, any technique known in the art that provides antibodies produced by continuous cell line cultures can be used. Examples include

various techniques, such as those in Kohler, G. and Milstein, C., Nature 256: 495-497 (1975); Kozbor et al., Immunology Today 4: 72 (1983); Cole et al., pg. 77-96 in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc. (1985).

Where the novel CD163 polypeptides are prepared without a tag attached, and
5 no antibodies are available, other well-known procedures for purification can be used. Such procedures include, without limitation, ion exchange chromatography, molecular sieve chromatography, HPLC, native gel electrophoresis in combination with gel elution, and preparative isoelectric focusing ("Isoprime" machine/technique, Hoefer Scientific). In some cases, two or more of these techniques may be combined
10 to achieve increased purity.

It should be understood that the definition of polypeptides of the invention is intended to include polypeptides bearing modifications other than insertion, deletion, or substitution of amino acid residues. By way of example, the modifications may be covalent in nature, and include for example, chemical bonding with polymers, lipids,
15 other organic, and inorganic moieties.

Antibodies

Also comprehended by the present invention are antibodies (e.g., monoclonal and polyclonal antibodies, single chain antibodies, chimeric antibodies, bifunctional/bispecific antibodies, humanized antibodies, human antibodies, and
20 complementary determining region (CDR)-grafted antibodies, including compounds which include CDR sequences which specifically recognize a polypeptide of the invention) specific for novel CD163 or fragments thereof.

The term "specific for," when used to describe antibodies of the invention, indicates that the variable regions of the antibodies of the invention recognize and
25 bind CD163s polypeptides exclusively (i.e., able to distinguish CD163s polypeptides from other known polypeptides by virtue of measurable differences in binding affinity, despite the possible existence of localized sequence identity, homology, or similarity between the novel CD163 and such polypeptides). It will be understood that specific antibodies may also interact with other proteins (for example, S. aureus protein A or
30 other antibodies in ELISA techniques) through interactions with sequences outside the variable region of the antibodies, and in particular, in the constant region of the molecule. Screening assays to determine binding specificity of an antibody of the invention are well known and routinely practiced in the art. For a comprehensive

discussion of such assays, see Harlow et al. (Eds), Antibodies A Laboratory Manual; Cold Spring Harbor Laboratory; Cold Spring Harbor, NY (1988), Chapter 6.

Antibodies that recognize and bind fragments of the CD163s polypeptides of the invention are also contemplated, provided that the antibodies are, first and foremost, specific for novel CD163 polypeptides. Antibodies of the invention can be produced using any method well known and routinely practiced in the art. Non-human antibodies may be humanized by any methods known in the art. In one method, the non-human CDRs are inserted into a human antibody or consensus antibody framework sequence. Further changes can then be introduced into the antibody framework to modulate affinity or immunogenicity.

Antibodies of the invention are useful for, diagnostic purposes to detect or quantitate CD163s, as well as purification of CD163s. Kits comprising an antibody of the invention for any of the purposes described herein are also comprehended. In general, a kit of the invention also includes a control antigen for which the antibody is immunospecific

The present invention is further illustrated, but not limited, by the following examples.

Example 1: Transient transfection with porcine CD163 confers permissivity to PRRS virus infection to a non-permissive cell line. Total mRNA from primary porcine alveolar macrophage cells was used to construct a cDNA library in the plasmid pCMV-Sport6.1 (Invitrogen), with the cDNA cloned between the *EcoRV* and *NotI* sites. A member of this library, when isolated and transiently transfected into the BHK-21 (baby hamster kidney) cell line, conferred a PRRS-permissive phenotype. Cells were grown in Dulbecco's modified Eagle medium (DMEM) supplemented with 5% fetal bovine serum (FBS) in a 5% CO₂ atmosphere at 37°C. Cell cultures were transiently transfected using 10.0 uL of Lipofectamine 2000 (Invitrogen) and 2.0ug of plasmid. A duplicate monolayer was transfected with negative control plasmid pPAMB. This plasmid is pCMV-Sport6.1 lacking an insert. Transfection efficiency was monitored with a plasmid expressing green fluorescent protein (GFP). Approximately 24 hours post-transfection, monolayers were infected with either North American (isolate P129) or European (isolate 96V198) genotypes of PRRS virus. For detection of PRRS replication, the monolayers were fixed using 80% acetone approximately 24 hours post-infection and incubated for approximately 1 hour with

FITC-conjugated monoclonal antibody SDOW17 (Rural Technologies Inc.). This monoclonal antibody is specific for PRRS viral nucleocapsid expressed from open reading frame 7. A Nikon TE 300 inverted fluorescent microscope with a 10x objective was used to photograph a monolayer containing FITC positive cells and a negative control monolayer.

It was confirmed that transfected cells became permissive to both the North American (isolate P129) and European (isolate 96V198) genotypes of PRRSV. Expression of viral genes could be detected in many of the transfected BHK cells, and progeny virus was readily detectable in the supernatant. Control transfections using vector without insert or irrelevant plasmids did not confer permissivity.

Sequencing of the insert in the functional plasmid, using the Big Dye Terminator Version 1.0 Sequence Reaction kit (Applied Biosystems, Foster City, CA) and the Applied Biosystems 3730 DNA Analyzer (Applied Biosystems), revealed a gene that was highly homologous to the published porcine CD163 gene cDNA (Genbank accession number AJ311716). The cDNA we identified contained additional 5' and 3' untranslated regions relative to AJ311716, and the open reading frame differed in three ways: (1) a 738 bp internal deletion near the 5' end, (2) a 15 bp extension of the 5' end to an upstream ATG codon, and (3) sixteen nucleotide changes predicted to cause 10 amino acid changes. Nucleotide sequence identity between the sequences was 99.4%. Alignments of the newly discovered porcine CD163 sequence with the previously reported sequence AJ311716 are shown in figures 1 and 2. The novel porcine CD163 variant was designated "susCD163v1".

SEQUENCE							ID NO
							SEQ ID NO:5
gtaataatac	aagaagat	aaatgggcat	aaaaccttgg	aatggacaaa	ctcagaatgg	60	
tgctacatga	aaactctgga	tctgcagacc	tgaaactgag	agtggtagat	ggagtcactg	120	
aatgttcagg	aagattggaa	gtgaaattcc	aaggagaatg	gggaacaatc	tgtgatgatg	180	
gctgggatag	tgatgatgcc	gctgtggcat	gtaagcaact	gggatgtcca	actgctgtca	240	
ctgccattgg	tcgagttaac	gccagtgagg	gaactggaca	catttggctt	gacagtgttt	300	
cttgccatgg	acacgagtct	gctctctggc	agtgtagaca	ccatgaatgg	ggaaagcatt	360	
attgcaatca	taatgaagat	gctggtgtga	catgttctga	tggatcagat	ctggaactga	420	
gacttaaagg	tggaggcagc	cactgtgctg	ggacagtggg	ggtggaaatt	cagaaactgg	480	
taggaaaagt	gtgtgataga	agctggggac	tgaaagaagc	tgatgtgggt	tgcaggcagc	540	
tgggatgtgg	atctgcactc	aaaacatcat	atcaagttta	ttccaaaacc	aaggcaacaa	600	
acacatggct	gtttgtaagc	agctgtaatg	gaaatgaaac	ttctcttttg	gactgcaaga	660	
attggcagtg	gggtggactt	agttgtgatc	actatgacga	agccaaaatt	acctgctcag	720	
cccacaggaa	accaggctg	gttggagggg	acattccctg	ctctggtcgt	gttgaagtac	780	

aacatggaga cacgtggggc accgtctgtg attctgactt ctctctggag gcggccagcg	840	
tgctgtgcag ggaactacag tgccgcactg tggtttccct cctgggggga gctcactttg	900	
gagaaggaag tggacagatc tgggctgaag aattccagtg tgaggggcac gagtcccacc	960	
tttactctg cccagtagca ccccgccctg acgggacatg tagccacagc agggacgtcg	1020	
gcgtagtctg ctcaagatac acacaaatcc gcttggtgaa tggcaagacc ccatgtgaag	1080	
gaagagtgga gctcaacatt cttgggtcct ggggggtccct ctgcaactct cactgggaca	1140	
tggaagatgc ccatgtttta tgccagcagc ttaaagtgg agttgccctt tctatcccgg	1200	
gaggagcacc ttttgggaaa ggaagtgagc aggtctggag gcacatgttt cactgcactg	1260	
ggactgagaa gcacatggga gattgttccg tctactgctct gggcgcatca ctctgttctt	1320	
cagggcaagt ggccctctgta atctgctcag ggaaccagag tcagacacta tccccgtgca	1380	
attcatcatc ctccgaccca tcaagctcta ttatttcaga agaaaatggg gttgcctgca	1440	
tagggagtgg tcaacttcgc ctggctgatg gaggtggctg ttgtgctggg agagtagagg	1500	
tctatcatga gggctcctgg ggcaccatct gtgatgacag ctgggacctg aatgatgccc	1560	
atgtggtgtg caaacagctg agctgtggat gggccattaa tgccactggg tctgctcatt	1620	
ttggggaagg aacagggccc atttggctgg atgagataaa ctgtaatgga aaagaatctc	1680	
atatttggca atgccactca catggttggg ggccggcaca ttgcaggcat aaggaggatg	1740	
caggagtcac ctgctcggag ttcattgtctc tcagactgat cagtgaaaac agcagagaga	1800	
cctgtgcagg gcgcctggaa gttttttaca acggagcttg gggcagcgtt ggcaagaata	1860	
gcatgtctcc agccacagtg ggggtggat gcaggcagct gggctgtgca gacagagggg	1920	
acatcagccc tgcattctca gacaagacag tgtccaggca catgtgggtg gacaatgttc	1980	
agtgtcctaa aggacctgac accctatggc agtgcccatc atctccatgg aagaagagac	2040	
tggccagccc ctccagaggag acatggatca catgtgcaa caaaataaga cttcaagaag	2100	
gaaacactaa ttgttctgga cgtgtggaga tctggtacgg aggttcctgg ggcactgtgt	2160	
gtgacgactc ctgggacctt gaagatgctc aggtggtgtg ccgacagctg ggctgtggct	2220	
cagctttgga ggcaggaaaa gaggccgcat ttggccaggg gactgggccc atatggetca	2280	
atgaagtga gtgcaagggg aatgaaacct ccttgtggga ttgtcctgcc agatcctggg	2340	
gccacagtga ctgtggacac aaggaggatg ctgctgtgac gtgttcagaa attgcaaaga	2400	
gccgagaatc cctacatgcc acaggtcgct catcttttgt tgcacttgca atctttgggg	2460	
tcattctgtt ggctgtctc atcgattcc tcatttggac tcagaagcga agacagaggc	2520	
agcggctctc agttttctca ggaggagaga attctgtcca tcaaattcaa taccgggaga	2580	
tgaattcttg cctgaaagca gatgaaacgg atatgctaaa tccctcagga gaccactctg	2640	
aagtacaatg aaaaggaaaa tgggaattat aacctgggtg gttcagcctt taagatacct	2700	
tgatgaagac ctggactatt gaatgagcaa gaatctgcct cttacactga agattacaat	2760	
acagtcctct gtctcctggg attccaaaga ctgctgttga atttctaaaa aatagattgg	2820	
tgaatgtgac tactcaaagt tgtatgtaag actttcaagg gcattaaata aaaaagaata	2880	
ttgctgaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa	2930	
1 M D K L R M V L H E N S G S A D L K L R		SEQ ID NO:1 and 2
1 atggacaaactcagaatgggtgctacatgaaaactctggatctgcagacctgaaactgaga		
21 V V D G V T E C S G R L E V K F Q G E W		
61 gtggtagatggagtcactgaatgttcaggaagattggaagtgaattccaaggagaatgg		
41 G T I C D D G W D S D D A A V A C K Q L		
121 ggaacaatctgtgatgatggctgggatagtgatgatgccgctgtggcatgtaagcaactg		
61 G C P T A V T A I G R V N A S E G T G H		
181 ggatgtccaactgctgtcactgccattggctcgagttaacgccagtgaggggaactggacac		
81 I W L D S V S C H G H E S A L W Q C R H		

241	atttggcttgacagtgtttcttgccatggacacagagtctgctctctggcagtgtagacac	
101	H E W G K H Y C N H N E D A G V T C S D	
301	catgaatggggaaagcattattgcaatcataatgaagatgctgggtgtgacatgttctgat	
121	G S D L E L R L K G G G S H C A G T V E	
361	ggatcagatctggaactgagacttaaggtggaggcagccactgtgctgggacagtggag	
141	V E I Q K L V G K V C D R S W G L K E A	
421	gtggaaattcagaaactggtaggaaaagtgtgtgatagaagctggggactgaaagaagct	
161	D V V C R Q L G C G S A L K T S Y Q V Y	
481	gatgtggtttgacaggcagctgggatgtggatctgcactcaaacatcatatcaagtttat	
181	S K T K A T N T W L F V S S C N G N E T	
541	tccaaaaccaaggcaacaaacacatggctgtttgtagcagctgtaatggaaatgaaact	
201	S L W D C K N W Q W G G L S C D H Y D E	
601	tctctttgggactgcaagaattggcagtgggggtggacttagttgtgatcactatgacgaa	
221	A K I T C S A H R K P R L V G G D I P C	
661	gccaaaattacctgctcagcccacaggaaacccaggctgggtggaggggacattccctgc	
241	S G R V E V Q H G D T W G T V C D S D F	
721	tctggctcgtgttgaagtacaacatggagacacgtggggcaccgtctgtgattctgacttc	
261	S L E A A S V L C R E L Q C G T V V S L	
781	tctctggaggcggccagcgtgctgtgcagggaactacagtgcggcactgtggtttccctc	
281	L G G A H F G E G S G Q I W A E E F Q C	
841	ctgggggggagctcacttttgagaaggaagtggacagatctgggctgaagaattccagtgt	
301	E G H E S H L S L C P V A P R P D G T C	
901	gaggggcacgagtcccacctttcactctgccagtagcaccgccctgacgggacatgt	
321	S H S R D V G V V C S R Y T Q I R L V N	
961	agccacagcaggacgtcggcgtagtctgctcaagatacacacaaatccgcttggtgaat	
341	G K T P C E G R V E L N I L G S W G S L	
1021	ggcaagaccccatgtgaaggaagagtggagctcaacattcttgggtcctgggggtccctc	
361	C N S H W D M E D A H V L C Q Q L K C G	
1081	tgcaactctcactgggacatggaagatgccatgttttatgccagcagcttaaatgtgga	
381	V A L S I P G G A P F G K G S E Q V W R	
1141	gttgccctttctatcccgggaggagcaccttttgggaaaggaagtgagcaggtctggagg	
401	H M F H C T G T E K H M G D C S V T A L	
1201	cacatgtttcactgcactgggactgagaagcacatgggagattgttccgtcactgctctg	
421	G A S L C S S G Q V A S V I C S G N Q S	
1261	ggcgcacactctgttcttcaggggcaagtggcctctgtaatctgctcagggaaccagagt	
441	Q T L S P C N S S S S D P S S S I I S E	
1321	cagacactatccccgtgcaattcatcctcggacccatcaagctctattatttcagaa	
461	E N G V A C I G S G Q L R L V D G G G R	
1381	gaaaatggtgttgctgcatagggagtggtcaacttcgcctggctcgatggaggtggtcgt	
481	C A G R V E V Y H E G S W G T I C D D S	
1441	tgtgctgggagagtagaggtctatcatgagggtcctggggcaccatctgtgatgacagc	
501	W D L N D A H V V C K Q L S C G W A I N	
1501	tgggacctgaatgatgcccatgtgtgtgcaaacagctgagctgtggatgggccattaat	
521	A T G S A H F G E G T G P I W L D E I N	
1561	gccactgggtctgctcattttggggaaggaacaggggccatttggctggatgagataaac	
541	C N G K E S H I W Q C H S H G W G R H N	
1621	tgtaatggaaaagaatctcatattttggcaatgccactcacatgggttgggggaggcacaat	
561	C R H K E D A G V I C S E F M S L R L I	
1681	tgacagcagaaaggagatgcaggagtcactgctcggagttcatgtctctcagactgatc	
581	S E N S R E T C A G R L E V F Y N G A W	
1741	agtgaaaacagcagagagacctgtgcaggggcctggaagtgttttacaacggagcttgg	
601	G S V G K N S M S P A T V G V V C R Q L	
1801	ggcagcgttggcaagaatagcatgtctccagccacagtgggggtggtatgcaggcagctg	
621	G C A D R G D I S P A S S D K T V S R H	
1861	ggctgtgcagacagaggggacatcagccctgcacttccagacaagacagtgtccaggcac	
641	M W V D N V Q C P K G P D T L W Q C P S	
1921	atgtgggtggacaatgttcagtgtcctaaaggacctgacaccctatggcagtgcccatca	
661	S P W K K R L A S P S E E T W I T C A N	
1981	tctccatggaagaagagactggccagccctcagaggagacatggatcacatgtgccaac	
681	K I R L Q E G N T N C S G R V E I W Y G	
2041	aaaataagacttcaagaaggaacactaattgttctggacgtgtggagatctggtacgga	
701	G S W G T V C D D S W D L E D A Q V V C	
2101	ggttccctggggcactgtgtgtgacgactcctgggaccttgaagatgctcaggtggtgtgc	
721	R Q L G C G S A L E A G K E A A F G Q G	
2161	cgacagctgggctgtggctcagctttggaggcaggaaaagaggccgcatattggccagggg	
741	T G P I W L N E V K C K G N E T S L W D	
2221	actgggcccataatggctcaatgaagtgaagtgaaggggaatgaaacctccttggggat	
761	C P A R S W G H S D C G H K E D A A V T	
2281	tgtcctgccagatcctggggccacagtgactgtggacacaaggaggatgctgctgtgacg	
781	C S E I A K S R E S L H A T G R S S F V	
2341	tgttcagaaattgcaaagagccgagaatccctacatgccacaggtcgctcatcttttgtt	
801	A L A I F G V I L L A C L I A F L I W T	
2401	gcacttgcaatctttggggctcattctgttggcctgtctcatcgcattectcatttggact	
821	Q K R R Q R Q R L S V F S G G E N S V H	
2461	cagaagcgaagacagaggcagcggtctcagttttctcaggaggagagaattctgtccat	
841	Q I Q Y R E M N S C L K A D E T D M L N	
2521	caaattcaataccgggagatgaattcttgcctgaaagcagatgaaacggatatgctaaat	
861	P S G D H S E V Q	
2581	ccctcaggagaccactctgaagtacaa	
1	MDKLRMVLHE NSGSADLKL R VVDGVTECSG RLEVKFQGEW GTICDDGWDS	SEQ ID
51	DDAAVACKQL GCPTAVTAIG RVNASEGTGH IWLDSVSCGH HESALWQCRH	

64680-1737

101	HEWGKHYCNH	NEDAGVTCSD	GSDLELRKQ	GGSHCAGTVE	VBIQKLVGKY	NO:2
151	CDRSWGLKEA	DVVCROLGCG	SALKTSYQVY	SKTKATNTWL	FVSSCNGNET	
201	SLNDCIOWQW	GGLSCDHYDE	AKITCSAHRK	PLVGGDIPC	SGRVEVQHG	
251	TWGTVCDSDF	SLEAASVLCR	ELQCGTVVSL	LGGAHFGEGB	QQIWAEEFQC	
301	EGHESHLSLC	PVAPRPDQTC	SHSRDVGVVC	SRYTQIRLVN	GKTPCEGRVE	
351	LMILGSWQSL	CNEHMDMEDA	HVLCQQLKCG	VALSIPGGAP	FGKSEQVWR	
401	HMFCTGTTEK	HMGDCSVTAL	GASLCSSGQV	ASVICSQNGS	QTLSPCNSSS	
451	SDPSSSIISE	ENGVACIGSG	QLRLVDGGR	CAGRVEVYHE	GSNGTICDDS	
501	WDLNDARVVC	KQLSCGNAIN	ATGSAHFGE	TGPIWLDEIN	CNGKESHIWQ	
551	CHSHGWQRHM	CRHKEDAGVI	CSEFMSLRLI	SENSRETCAG	RLEVFFYNGAN	
601	GSVGKNSMSP	ATVGVVCRQL	GCADRGDISP	ASBDKTVERH	MMVDNVQCPK	
651	GPDILMQCPS	SPWKKRLASP	SEETWITCAN	KIRLQEGNTN	CSGRVEIWYG	
701	GSNGTVCCDS	WDLKDAQVVC	RQLGCGSALE	AGKEAAPGQG	TGPIWLNEVK	
751	CKGNETSLMD	CPARENGHED	CGHKEDAAVT	CSEIAKSRRES	LHATGRSSPV	
801	ALAIQGVILL	ACLIAFLIWT	QKRRQRQLS	VPSGGENSEVE	QIQYREMNQC	
851	LKADETDNLN	PSGDHSEVQ				

Example 2: Construction of plasmid pCMVsusCD163v1

Construction of the plasmid pCMVsusCD163v1 was performed as follows. The functional clone identified in the primary porcine macrophage cDNA library as

5 conferring PRRSV permissivity served as template for PCR amplification of the CD163 insert, including the 5' and 3' untranslated regions, using the primers 5'DS-CD163 (SEQ ID NO:6) (5'-CGGAATTCCGCGGATGTAATAATACAAGAAGA-3') and 3'CD163 (SEQ ID NO:7) (5'CCGCTCGAGTAGTCCAGGTCTTCATCAAGGTATCCTT-3'). Primer 5'DS-

10 CD163 incorporates a *Sac*II restriction site at the 5' end of the CD163 insert, while primer 3'CD163 incorporates an *Xho*I restriction site at the 3' end of the insert (underlined). Reactions containing 190ng of plasmid template were amplified using Platinum *Pfx* DNA polymerase (Invitrogen^{*} cat # 11708-013) following the manufacture's instructions. Reactions were heated to 94° for 2 minutes then cycled

15 35 times through 94° for 20 seconds, 55° for 30 seconds, and 68° for 3.5 minutes followed by a terminal extension at 72° for 7 minutes. The resulting PCR products were purified using the Qiaquick PCR purification kit (Qiagen cat # 28104), digested with restriction enzymes *Sac*II and *Xho*I, and the resulting fragments were gel purified using the Qiaquick Gel Extraction kit (Qiagen^{*} cat # 28704). The CD163 PCR

20 fragment was then ligated into the plasmid pCMV-Script (Stratagene cat # 212220) prepared to accept the digested PCR fragment by digestion with *Sac*II and *Xho*I followed by gel purification as described above. The ligated material was transformed into *E. coli* strain DH5α and recombinants were selected by growth in 50μg/ml kanamycin and identified by restriction analysis. The resulting plasmid,

25 "pCMVsusCD163v1", contains the internally deleted porcine CD163 insert described in Example 1 under the transcriptional control of the eukaryotic CMV promoter and

^{*}Trade-mark

64680-1737

the neomycin/kanamycin resistance gene under the control of both eukaryotic and prokaryotic promoters.

Example 3: Construction of the pRSV-Script expression vector and pRSVsusCD163v1

5 The plasmid pRc/RSV (Invitrogen) was used as a template for PCR amplification of the RSV promoter. RSV promoter sequence was contained within nucleotides 209 through 604 of pRc/RSV. Forward primer PCIRSVLTR (SEQ ID NO:8) (5'-ACACTCGACATGTCGATGTACGGGCCAGATATACGCGT-3') and reverse primer VSRRTLSAC (SEQ ID NO: 9)

10 (5'TTCCTTACAGAGCTCGAGGTGCACACCAATGTGGTGAA -3') were synthesized. Restriction endonuclease *Pci* I and *Sac* I recognition sites (underlined) were incorporated into the 5' and 3' primers, respectively, for future cloning. PCR was performed using the HotMaster Taq DNA Polymerase kit (Eppendorf) following the manufacturer's instructions. The reactions contained 0.9ng of pRc/RSV plasmid

15 template and 0.3μM of each primer described above. The reactions were heated to 94° for 2 minutes then cycled 30 times through 94° for 20 seconds, 52° for 10 seconds, and 65° for 1 minute. The resulting PCR fragment was digested with restriction enzymes *Pci* I and *Sac* I, gel purified, and cloned into the plasmid pCMV-Script (Stratagene) that had been similarly digested to remove the CMV promoter

20 sequence. The final construct placed the RSV promoter immediately upstream of the multiple cloning site, and was named "pRSV-Script".

 The susCD163v1 insert was cloned behind the RSV promoter as follows. The susCD163v1 sequence was excised from plasmid pCMVsusCD163v1 by restriction digestion (*Kpn* I and *Sac* II) and gel purified. This fragment was ligated into pRSV-

25 Script which had also been digested with the same enzymes and gel purified. The ligation mixture was transformed into DH5α *E. coli* and transformants selected using kanamycin at 50μg/ml. The clone contained the correct insert was designed "pRSVsusCD163v1".

Example 4: Cloning and characterization of a longer variant of porcine CD163 cDNA

30

 Based on the porcine CD163v1 sequence, a forward primer 5'CD163NotIIlong (SEQ ID NO:10)

(5'CGGTCCGGAGCGGCCGCGATGTAATAATACAAGAAGATTAAATGG-3') and a reverse primer 3'CD163KpnI (SEQ ID NO:11)

*Trade-mark

64680-1737

(5'CGGTTGGTACCCAGCAATATTCTTTTTTATTTAATGCC-3') were designed using the Lasergene PrimerSelect program (DNASTAR Inc., Madison WI) for amplification of a full-length porcine CD163 gene. Restriction endonuclease sites for *Not* I and *Kpn* I (underlined) were included in 5' and 3' primers, respectively, to allow for convenient cloning. Total cellular RNA was prepared from primary alveolar macrophages (PAM) harvested from lung lavages of healthy pigs. RNA preparation was done using the RNeasy mini kit (Qiagen, Valencia, CA). RT-PCR reactions were prepared using the SuperScript one-step RT-PCR for Long Templates kit (Invitrogen, Carlsbad, CA) and RT-PCR parameters were set as follows: 50°C for 30 min, 94°C for 2 min, (94°C 30 sec, 55°C 30 sec and 68°C 4 min) for 35 cycles, 72°C for 10 min. PCR products were analyzed on 0.8% SeaKem^{*} GTG agarose gels. RT-PCR products of various sizes were cut from agarose gels and DNA was extracted using the GeneClean kit (QBiogene). These RT-PCR products were cloned into the pCR2.1-TOPO cloning vector (Invitrogen). Clones were analyzed by restriction enzyme digestion for the presence of an insert. Colonies containing inserts were sequenced using Big Dye Terminator Version 1.0 Sequence Reaction kit (Applied Biosystems, Foster City, CA) and Applied Biosystems 3730 DNA Analyzer (Applied Biosystems) to confirm sequence authenticity. Sequences were edited and assembled using the Lasergene EditSeq and SeqMan programs (DNASTAR Inc., Madison WI). One plasmid with a large insert was designated "pCRsusCD163v2" (pCR2.1 containing porcine CD163 variant 2 which we have designated SEQ ID NO:12). The coding sequence contained within SEQ ID NO:12 is reproduced below and is designated SEQ ID NO:13. Sequence analysis showed that this porcine CD163 encodes an amino acid sequence of 1115 amino acids which we have designated SEQ ID NO:14. When compared to the porcine CD163 sequence in GenBank (Accession No. AJ311716), our CD163v2 sequence is 98.9% identical at the amino acid level. CD163v2 also has an additional 5 amino acid residues at the extreme 5' end, extending the open reading frame to an in-frame upstream ATG initiation codon (as in the porcine CD163v1 sequence described in example 1). Porcine CD163 is 84.3% identical to human CD163 (GenBank Accession No. Z22968), and 73.7% identical to mouse CD163 (GenBank Accession No. AF274883) at the amino acid level. The predicted signal sequence and transmembrane region of SEQ ID NO:14 are indicated by underlining

*Trade-mark

and bolding respectively. To determine whether other CD163 sequences contain similar sequence features is easily determined by inspection of the sequence.

SEQUENCE	ID NO
gtaataatac aagaagattt aaatggcata aaaccttga atggacaaac tcagaatggt 60 gctacatgaa aactctggat ctgcagactt tagaagatgt tctgcccatt taagttcctt 120 cacttttgct gtagtcgctg ttctcagtgc ctgcttggtc actagttctc ttggaggaaa 180 agacaaggag ctgaggctaa cgggtggtga aaacaagtgc tctggaagag tggagggtgaa 240 agtgcaggag gagtggggaa ctgtgtgtaa taatggctgg gacatggatg tggctctctgt 300 tgtttgtagg cagctgggat gtccaactgc tatcaaagcc actggatggg ctaatttttag 360 tgcaggttct ggacgcattt ggatggatca tgtttcttgt cgagggaatg agtcagctct 420 ctgggactgc aaacatgatg gatggggaaa gcataactgt actcaccaac aggatgctgg 480 agtaacctgc tcagatggat ctgatttaga gatggggctg gtgaatggag gaaaccggtg 540 cttaggaaga atagaagtca aatttcaagg acggtgggga acagtgtgtg atgataactt 600 caacataaat catgcttctg tggtttgtaa acaacttgaa tgtggaagtg ctgtcagttt 660 ctctggttca gctaattttg gagaagggtc tggaccaatc tggtttgatg atcttgtatg 720 caatggaaat gagtcagctc tctggaactg caaacatgaa ggatggggaa agcacaattg 780 cgatcatgct gaggatgctg gagtgatttg cttaaattga gcagacctga aactgagagt 840 ggtagatgga gtcactgaat gttcaggaag attggaagtg aaattccaag gagaatgggg 900 aacaatctgt gatgatggct gggatagtga tgatgccgt gtggcatgta agcaactggg 960 atgtccaact gctgtcactg ccattggctg agttaacgcc agtgaggga ctggacacat 1020 ttggcttgac agtgtttctt gccatggaca cgagtctgct ctctggcagt gtagacacca 1080 tgaatgggga aagcattatt gcaatcatga tgaagatgct ggtgtgacat gttctgatgg 1140 atcagatctg gaactgagac ttaaagggtg aggcagccac tgtgctggga cagtggaggt 1200 ggaaattcag aaactggtag gaaaagtgtg tgatagaagc tggggactga aagaagctga 1260 tgtggtttgc aggcagctgg gatgtggatc tgcactcaa acatcatatc aagtttattc 1320 caaaaccaag gcaacaaaca catggctgtt tgtaagcagc tgtaatggaa atgaaacttc 1380 tctttgggac tgcaagaatt ggcagtgggg tggacttagt tgtgatcact atgacgaagc 1440 caaaattacc tgctcagccc acaggaaacc caggctgggt ggaggggaca ttccctgctc 1500 tggctcgtgt gaagtacaac atggagacac gtggggcacc gtctgtgatt ctgacttctc 1560 tctggaggcg gccagcgtgc tgtgcaggga actacagtgc ggcactgtgg ttccctcct 1620 ggggggagct cactttggag aaggaagtgg acagatctgg gctgaagaat tccagtgtga 1680 ggggcacgag tcccaccttt cactctgccc agtagcacc cgccctgacg ggacatgtag 1740 ccacagcagg gacgtcggcg tagtctgctc aagatacaca caaatccgct tggatgaatg 1800 caagaccca tgtgaaggaa gagtggagct caacattctt gggctcctggg ggtccctctg 1860 caactctcac tgggacatgg aagatgcccc tgttttatgc cagcagctta aatgtggagt 1920 tgccctttct atcccgggag gagcaccttt tgggaaagga agtgagcagg tctggaggca 1980 catgtttcac tgcactggga ctgagaagca catgggagat tgttccgtca ctgctctggg 2040 cgcatcactc tgttcttcag ggcaagtggc ctctgtaatc tgctcaggga accagagtca 2100 gacactatct ccgtgcaatt catcatcctc ggacccatca agctctatta tttcagaaga 2160 aaatggtgtt gcctgcatag ggagtggtea acttcgctg gtogatggag gtggctcgtt 2220 tgctgggaga gtagaggtct atcatgaggg ctctggggc accatctgtg atgacagctg 2280	SEQ ID NO:12

ggacctgaat gatgcccattg tgggtgtgcaa acagctgagc tgtggatggg ccattaatgc 2340 cactgggttct gctcattttg gggaaggaac agggcccatt tggctggatg agataaactg 2400 taatggaaaa gaatctcata tttggcaatg ccaactcacat ggttgggggc ggcacaattg 2460 caggcataag gaggatgcag gagtcatctg ctacagagttc atgtctctga gactgatcag 2520 tgaaaacagc agagagacct gtgcagggcg cctggaagtt ttttacaacg gagcttgggg 2580 cagcgttggc aggaatagca tgtctccagc cacagtgggg gtggtatgca ggcagctggg 2640 ctgtgcagac agagggggaca tcagccctgc atcttcagac aagacagtgt ccaggcacat 2700 gtgggtggac aatgttcagt gtcctaaagg acctgacaca ctatggcagt gccatcatc 2760 tccatggaag aagagactgg ccagccctc agaggagaca tggatcacat gtgccaacaa 2820 aataagactt caagaaggaa aactaattg ttctggacgt gtggagatct ggtacggagg 2880 ttctggggc actgtgtgtg acgactcctg ggaccttgaa gatgctcagg tgggtgtgccg 2940 acagctgggc tgtggctcag ctttggaggc aggaaaagag gccgcatttg gccaggggac 3000 tgggcccata tggctcaatg aagtgaagtg caaggggaat gaaacctcct tgtgggattg 3060 tcctgccaga tcctggggcc acagtgactg tggacacaag gaggatgctg ctgtgacgtg 3120 ctacagaaatt gcaaagagcc gagaatccct acatgccaca ggtcgctcat cttttgttgc 3180 acttgcaatc tttgggtca ttctgttggc ctgtctcatc gcattcctca tttggactca 3240 gaagcgaaga cagaggcagc ggctctcagt tttctcagga ggagagaatt ctgtccatca 3300 aattcaatac cgggagatga attcttgctt gaaagcagat gaaacggata tgctaaatcc 3360 ctacaggagac cactctgaag tacaatgaaa aggaaaatgg gaattataac ctggtgagtt 3420 cagcctttaa gataccttga tgaagacctg gactattgaa tgagcaagaa tctgcctctt 3480 aactgaaga ttacaatata gtcctctgtc tcctgggtatt ccaaagactg ctgctgaatt 3540 tctaaagaat agattgggtga atgtgactac tcaaagttgt atgtaagact ttcaagggca 3600 ttaaataaaa aagaatattg ctg 3623	
1 M D K L R M V L H E N S G S A D F R R C 1 atggacaaactcagaatgggtgctacatgaaaactctggatctgcagactttagaagatgt 21 S A H L S S F T F A V V A L S A C L V 61 tctgcccatttaagttccttcaacttttgctgtagtcgctgttctcagtgccctgttggtc 41 T S S L G G K D K E L R L T G G E N K C 121 actagttctcttggaggaaaagacaaggagctgaggctaaccgggtgggtgaaaacaagtgc 61 S G R V E V K V Q E E W G T V C N N G W 181 tctggaagagtggaggtgaaagtgcaggaggagtggggaactgtgtgtaataatggctgg 81 D M D V V S V V C R Q L G C P T A I K A 241 gacatggatgtggtctctgttgtttgttaggcagctgggatgtccaactgctatcaaagcc 101 T G W A N F S A G S G R I W M D H V S C 301 actggatgggctaatttttagtgcaggttctggacgcatttggatggatcatgtttcttgt 121 R G N E S A L W D C K H D G W G K H N C 361 cgagggaatgagtcagctctctgggactgcaaacatgatggatggggaaagcataactgt 141 T H Q Q D A G V T C S D G S D L E M G L 421 actcaccaacaggatgctggagtaacctgctcagatggatctgatttagagatggggctg 161 V N G G N R C L G R I E V K F Q G R W G 481 gtgaatggaggaaaccgggtgcttaggaagaatagaagtcaaatttcaaggacgggtgggga 181 T V C D D N F N I N H A S V V C K Q L E 541 acagtgtgtgatgataacttcaacataaatcatgcttctgtggtttgtaaacaacttgaa 201 C G S A V S F S G S A N F G E G S G P I 601 tgtggaagtgtgtcagtttctctggttcagctaattttggagaagggttctggaccaatc 221 W F D D L V C N G N E S A L W N C K H E 661 tggtttgatgatcttgtatgcaatggaaatgagtcagctctctggaactgcaaacatgaa 241 G W G K H N C D H A E D A G V I C L N G 721 ggatggggaaagcacaattgcgatcatgctgaggatgctggagtgatttgcttaaatgga 261 A D L K L R V V D G V T E C S G R L E V 781 gcagacctgaaactgagagtggtagatggagtcactgaatgttcaggaagattggaagtg 281 K F Q G E W G T I C D D G W D S D D A A 841 aaattccaaggagaatggggaacaatctgtgatgatggctgggatagtgatgatgccgct 301 V A C K Q L G C P T A V T A I G R V N A 901 gtggcatgtaagcaactgggatgtccaactgctgtcactgccattggctcgagttaacgcc 321 S E G T G H I W L D S V S C H G H E S A 961 agtgagggaactggacacatttggcttgacagtgtttcttgccatggacacgagtctgct	SEQ ID NO:13 and14

341	L W Q C R H H E W G K H Y C N H D E D A	
1021	ctctggcagtgtagacacccatgaatggggaaagcattattgcaatcatgatgaagatgct	
361	G V T C S D G S D L E L R L K G G G S H	
1081	ggtgtgacatgttctgatggatcagatctggaactgagacttaaagggtggaggcagccac	
381	C A G T V E V E I Q K L V G K V C D R S	
1141	tgtgctgggacagtggaggtggaaattcagaaactggtaggaaaagtgtgtgatagaagc	
401	W G L K E A D V V C R Q L G C G S A L K	
1201	tggggactgaaagaagctgatgtgtgtttgcaggcagctgggatgtggatctgcactcaaa	
421	T S Y Q V Y S K T K A T N T W L F V S S	
1261	acatcatatcaagtttattccaaaaccaaggcaacaacacatggctgtttgtgaagcagc	
441	C N G N E T S L W D C K N W Q W G G L S	
1321	tgtaatggaaatgaaacttctctttgggactgcaagaattggcagtggggtggacttagt	
461	C D H Y D E A K I T C S A H R K P R L V	
1381	tgtgatcactatgacgaagccaaaattacctgctcagcccacaggaaaccaggctggtt	
481	G G D I P C S G R V E V Q H G D T W G T	
1441	ggaggggacattccctgctctggtcgtgttgagtacaacatggagacacgtggggcacc	
501	V C D S D F S L E A A S V L C R E L Q C	
1501	gtctgtgattctgacttctctctggaggcggccagcgtgctgtgcagggaactacagtgc	
521	G T V V S L L G G A H F G E G S G Q I W	
1561	ggcactgtggtttccctcctggggggagctcactttggagaaggaagtggacagatctgg	
541	A E E F Q C E G H E S H L S L C P V A P	
1621	gctgaagaattccagtgtgaggggcacgagtcacacctttcactctgccagtagcaccc	
561	R P D G T C S H S R D V G V V C S R Y T	
1681	cggcctgacgggacatgtagccacagcaggacgctcggcgtagtctgctcaagatacaca	
581	Q I R L V N G K T P C E G R V E L N I L	
1741	caaatccgcttggtgaatggcaagaccccatgtgaaggaagagtggagctcaacattctt	
601	G S W G S L C N S H W D M E D A H V L C	
1801	gggtcctgggggtccctctgcaactctcactgggacatggaagatgcccattgttttatgc	
621	Q Q L K C G V A L S I P G G A P F G K G	
1861	cagcagcttaaagtgtggagttgccctttctatcccgggaggagcaccttttgggaaagga	
641	S E Q V W R H M F H C T G T E K H M G D	
1921	agtgagcaggtctggaggcacatgtttcactgcactgggactgagaagcacatgggagat	
661	C S V T A L G A S L C S S G Q V A S V I	
1981	tgttcgctcactgctctgggcgcacatcactctgttcttcaggggcaagtggcctctgtaac	
681	C S G N Q S Q T L S P C N S S S S D P S	
2041	tgctcagggaaaccagagtcagacactatctccgtgcaattcatcatcctcggacccatca	
701	S S I I S E E N G V A C I G S G Q L R L	
2101	agctctattatttcagaagaaaatggtgttgctgcatagggagtgggtcaacttcgctg	
721	V D G G G R C A G R V E V Y H E G S W G	
2161	gtcgtgaggggtggtcgttgtgctgggagagtagaggtctatcatgagggctcctggggc	
741	T I C D D S W D L N D A H V V C K Q L S	
2221	accatctgtgatgacagctgggacctgaatgatgcccattgtgtgtgcaaacagctgagc	
761	C G W A I N A T G S A H F G E G T G P I	
2281	tgtggatgggcccattaatgccactgggttctgctcattttggggaaggaacagggccatt	
781	W L D E I N C N G K E S H I W Q C H S H	
2341	tggctggatgagataaactgtaatggaaaagaatctcatatttggcaatgccactcacat	
801	G W G R H N C R H K E D A G V I C S E F	
2401	ggttggggggcggcacaattgcaggcataaggaggatgcaggagtcactctgctcagagttc	
821	M S L R L I S E N S R E T C A G R L E V	
2461	atgtctctgagactgatcagtgaaaacagcagagagacctgtgcagggcgcttgaagtt	
841	F Y N G A W G S V G R N S M S P A T V G	
2521	ttttacaacggagcttggggcagcgttggcaggaatagcatgtctccagccacagtgggg	
861	V V C R Q L G C A D R G D I S P A S S D	
2581	gtggtatgcaggcagctgggctgtgcagacagaggggacatcagccctgcatcttcagac	
881	K T V S R H M W V D N V Q C P K G P D T	
2641	aagacagtgtccaggcacatgtgggtggacaatgttcagtgtcctaaggacctgacaca	
901	L W Q C P S S P W K K R L A S P S E E T	
2701	ctatggcagtgcccattctccatgggaagaagactggccagcccctcagaggagaca	
921	W I T C A N K I R L Q E G N T N C S G R	
2761	tggatcacatgtgccaacaaaataagacttcaagaaggaaacactaattgttctggacgt	
941	V E I W Y G G S W G T V C D D S W D L E	
2821	gtggagatctggtacggaggttccctggggcactgtgtgtgacgactcctgggaccttgaa	
961	D A Q V V C R Q L G C G S A L E A G K E	
2881	gatgctcaggtggtgtgccgacagctgggctgtggctcagctttggaggcaggaaaagag	
981	A A F G Q G T G P I W L N E V K C K G N	
2941	gccgcatttggccaggggactggggcccatatggctcaatgaagtgaagtgaaggggaat	
1001	E T S L W D C P A R S W G H S D C G H K	
3001	gaaacctccttgtgggattgtcctgccagatcctggggccacagtgactgtggacacaag	
1021	E D A A V T C S E I A K S R E S L H A T	
3061	gaggatgctgctgtgacgtgctcagaaattgcaaagagccgagaatccctacatgccaca	
1041	G R S S F V A L A I F G V I L L A C L I	
3121	ggtcgtcatcttttgttgacttgaatctttggggtcattctgttggcctgtctcatc	
1061	A F L I W T Q K R R Q R Q R L S V F S G	
3181	gcattcctcatttggactcagaagcgaagacagaggcagcggtctcagttttctcagga	
1081	G E N S V H Q I Q Y R E M N S C L K A D	
3241	ggagagaattctgtccatcaaattcaataccgggagatgaattcttgctgaaagcagat	
1101	E T D M L N P S G D H S E V Q	
3301	gaaacggatgtgctaaatccctcaggagaccactctgaagtacaa	
1	MDKLRMLHE NSGSADFRRS SAHLSSFTFA VVAVLSACLV TSSLGGKDKE	SEQ ID
51	LRLTGGENKC SGRVEVKVQE EWGTVCNNGW DMDVSVVCR QLCPTAIKA	

101	TGWANFSAGS	GRIWMDHVSC	RGNESALWDC	KHDGWGKHNC	THQQDAGVTC	NO:14
151	SDGSDLEMGL	VNGGNRCLGR	IEVKFQGRWG	TVCDDNFNIN	HASVVCKQLE	
201	CGSAVSFSGS	ANFGECSGPI	WFDDLVCNGN	ESALWNCKHE	GWGKHNCDDHA	
251	EDAGVICLNG	ADLKLVRVDG	VTECSGRLEV	KFQGEWGTIC	DDGWDSDDAA	
301	VACKQLGCPT	AVTAIGRVNA	SEGTGHIWLD	SVSCHGHESA	LWQCRHHEWG	
351	KHYCNHDEDA	GVTCSDGSDL	ELRLKGGGSH	CAGTVEVEIQ	KLVGKVCDS	
401	WGLKEADVVC	RQLGCGSALK	TSYQVYSKTK	ATNTWLFVSS	CNGNETSLWD	
451	CKNWQWGGLS	CDHYDEAKIT	CSAHRKPRLV	GGDIPCSGRV	EVQHGDWTGT	
501	VCDSDFSLEA	ASVLCRELQC	GTVVSLLGGA	HFGEGSGQIW	AEEFQCEGHE	
551	SHLSLCPVAP	RPDGTCSHSR	DVGVVCSRYT	QIRLVNGKTP	CEGRVELNIL	
601	GSWGS LCNSH	WDMEDAHVLC	QQLKCGVALS	IPGGAPFGKG	SEQVWRHMFH	
651	CTGTEKHMGD	CSVTALGASL	CSSGQVASVI	CSGNQSQTLS	PCNSSSSDPS	
701	SSIISEENG	ACIGSGQLRL	VDGGGRCAGR	VEVYHEGSWG	TICDDSWDLN	
751	DAHVVCKQLS	CGWAINATGS	AHFEGGTGPI	WLDEINCNGK	ESHIWQCHSH	
801	GWGRHNCRHK	EDAGVICSEF	MSLRLISENS	RETCAGRLEV	FYNGAWGSVG	
851	RNSMSPATVG	VVCRQLGCAD	RGDISPASSD	KTVSRHMMVD	NVQCPKGPDT	
901	LWQCPSSPWK	KRLASPSEET	WITCANKIRL	QEGNTNCSGR	VEIWYGGSWG	
951	TVCDDSWDLE	DAQVVCRQLG	CGSALEAGKE	AAFQQGTGPI	WLNEVKCKGN	
1001	ETSLWDCPAR	SWGHSDCGHK	EDAAVTCSEI	AKSRESLHAT	GRSSFVALAI	
1051	FGVILLACLI	AFLIWTQKRR	QRQLSVFSG	GENSVHQIQY	REMNSCLKAD	
1101	ETDMLNPSGD	HSEVQ				

Sus CD163 v2 in pCRsusCD163v2 was liberated from pCR2.1 vector after restriction enzymes *Kpn* I and *Not* I digestion and gel purification. Recipient vector pCMV-script was also cut with the same restriction enzyme pair and allowed for directional cloning of susCD163v2 into the pCMV-script. After ligation of susCD163 v2 with pCMV-script, the ligated mixture was used to transform STBL 2 *E. coli* cells (Invitrogen). One transformant was found to contain the CD163 gene by restriction enzyme digestion analysis and was designed pCMV-script susCD163v2 clone#3.

Example 5: Preparation of a RSV promoter based expression system by direct ligation and transfection method.

A non-cloning based procedure to generate microgram quantities of linear DNA suitable for use in generating stable cell lines expressing CD163 from an RSV promoter was developed (figure 4). The procedure involves the isolation and ligation of two pieces of DNA, one containing the neomycin gene and RSV promoter cassette derived from pRSV-script, and the other containing the susCD163v2 coding sequence from pCMVsusCD163v2. Vector plasmid pRSV-Script was linearized with *Dra*III upstream of the neomycin gene, and bunted with the Klenow fragment of *E. coli* DNA polymerase. This plasmid was then digested with *Not*I immediately downstream of the RSV promoter. The pCMVsusCD163v2 clone was digested in the vector sequence downstream of the CD163 insert with *Drd*I, and blunted with Klenow fragment of DNA polymerase. The CD163 coding sequence was liberated from the vector with a *Not*I located immediately upstream of the CD163 coding sequence. For each plasmid digestion the appropriate fragments were purified from agarose gels. A large-scale ligation reaction was performed as follows. Approximately 20 µg of each

DNA fragment was incubated in a volume of 600 μ L with 15 units of T4 DNA ligase. The reaction was incubated at room temperature for 20 minutes, at which time an aliquot was removed and the reaction frozen on dry ice. Agarose gel analysis of the aliquot revealed that a significant amount of non-ligated DNA remained, so another
5 15 units of ligase was added and incubated for another 10 minutes at room temperature. Following ligation, a linear piece of DNA containing all of the appropriate elements was purified by agarose gel electrophoresis. Ligation of the two DNA fragments via the cohesive *Not* I termini resulted in the placement of the 5' sequences of the CD163 gene downstream of the RSV promoter, allowing for directed
10 expression of CD163 in mammalian cells. Once isolated, the purified DNA was used to transfect various mammalian cell lines.

Example 6: Cloning and characterization of human CD163 cDNA

Based on a known human CD163 cDNA sequence (GenBank Accession No. BC051281), a forward primer Hu5'Not (SEQ ID NO: 15)
15 (5'CACCGCGGCCGCGAAGTTATAAATCGCCACCATGAGCAAACCTCAGAATGG-3') and a reverse primer Hu3'Kpn (SEQ ID NO: 16)
(5'-TGCTCCGGTACCTAGTCCAGGTCTTCATCAAGGTATCTTA-3') were designed using the PrimerSelect program. Restriction sites for *Not*I and *Kpn*I (underlined) were incorporated into the 5' and 3' primers, respectively, to facilitate
20 cloning into expression vectors. The sequence CACC was added to the 5' end of the 5' primer to allow directional cloning into the pCDNA3.1D/V5/His/TOPO vector (Cat. No. K49001, Invitrogen, see figure 6). Human CD163 cDNAs were amplified from RNA extracted from the U937 cell line after stimulated with phorbol 12-myristate 13-acetate (100ng/ml) for 3 days. Total cellular RNA was prepared using
25 the RNeasy kit (Qiagen). RT-PCR reactions and sequencing methods were the same as described in Example 4. PCR products were separated on 0.8% SeaKem agarose gel and extracted from the gel using the GeneClean kit. PCR products were cloned directionally into the pCDNA3.1D/V5/His/TOPO vector following the manufacturer's instructions. Two clones with large inserts were sequenced. Sequencing and
30 sequence analysis methods were described in Example 4. A clone with a correct insert was designed "pcDNA3.1D-humCD163v2" and we have designated the sequence of the insert SEQ ID NO: 17

The CD163 open reading frame in pCDNA3.1D-humCD163v2 is 1121 residues in length (designated SEQ ID NO: 18 which encodes SEQ ID NO:19 disclosed below), and is 100% identical to Genbank Z22968 (a human CD163 cDNA of the same length). Our human CD163v2 sequence is also 100% identical to

5 Genbank BC051281 and Z22969 (splice variants of human CD163) except that 42 nonhomologous residues in the two Genbank sequences replace the seven carboxy-terminal residues of our sequence. This difference is due to the presence of an 83-nucleotide exon in BC051281 and Z22969, and the resulting frame shift at the 3' end of the exon. (Law, S.K., Micklem, K.J., Shaw, J.M., Zhang, X.P., Dong, Y., Willis,

10 A.C. and Mason, D.Y. (1993) A new macrophage differentiation antigen which is a member of the scavenger receptor superfamily. European Journal of Immunology 23 (9), 2320-2325).

SEQUENCE			ID NO
atgagcaaac tcagaatggt gctacttgaa gactctggat ctgctgactt cagaagacat	60	SEQ ID NO:17	
tttgtcaacc tgagtcacct caccattact gtggtcttac ttctcagtgc ctgttttgtc	120		
accagttctc ttggaggaac agacaaggag ctgaggctag tggatggtga aaacaagtgt	180		
agcgggagag tggaagtga agtccaggag gagtggggaa cgggtgtgtaa taatggctgg	240		
agcatggaag cggctctctgt gatattgtaac cagctgggat gtccaactgc tatcaaagcc	300		
cctggatggg ctaattccag tgcaggttct ggacgcattt ggatggatca tgtttcttgt	360		
cgtgggaatg agtcagctct ttgggattgc aaacatgatg gatggggaaa gcatagtaac	420		
tgtactcacc aacaagatgc tggagtgacc tgctcagatg gatccaattt ggaaatgagg	480		
ctgacgcgtg gagggaaat atgtttctgga agaatagaga tcaaattcca aggacggtgg	540		
ggaacagtgt gtgatgataa cttcaacata gatcatgcat ctgtcatttg tagacaactt	600		
gaatgtggaa gtgctgtcag tttctctggg tcatctaatt ttggagaagg ctctggacca	660		
atctggtttg atgatcttat atgcaacgga aatgagtcag ctctctggaa ctgcaaacat	720		
caaggatggg gaaagcataa ctgtgaccat gctgaggatg ctggagtgat ttgctcaaag	780		
ggagcagatc tgagcctgag actggtagat ggagtcactg aatgttcagg aagattagaa	840		
gtgagattcc aaggggaatg ggggacaata tgtgatgacg gctgggacag ttacgatgct	900		
gctgtggcat gcaagcaact gggatgtcca actgccgtca cagccattgg tcgagttaac	960		
gccagtaagg gatttggaac catctggctt gacagcgttt cttgccaggg acatgaacct	1020		
gctgtctggc aatgtaaaca ccatgaatgg ggaaagcatt attgcaatca caatgaagat	1080		
gctggcgtga catgttctga tggatcagat ctggagctaa gacttagagg tggaggcagc	1140		
cgtgtgctg ggacagttga ggtggagatt cagagactgt tagggaaggt gtgtgacaga	1200		
ggctggggac tgaaagaagc tgatgtggtt tgcaggcagc tgggatgtgg atctgcactc	1260		
aaaacatctt atcaagtga ctccaaaatc caggcaacaa acacatggct gtttctaagt	1320		
agctgtaacg gaaatgaaac ttctcttttg gactgcaaga actggcaatg gggaggactt	1380		
acctgtgatc actatgaaga agccaaaatt acctgctcag cccacaggga acccagactg	1440		
gttggagggg acattccctg ttctggacgt gttgaagtga agcatggtga cacgtggggc	1500		
tccatctgtg attcggactt ctctctggaa gctgccagcg ttctatgcag ggaattacag	1560		

tgtggcacag ttgtctctat cctgggggga gctcactttg gagagggaaa tggacagatc 1620	
tgggctgaag aattccagtg tgaggacat gagtcccatc tttcactctg cccagtagca 1680	
ccccgcccag aaggaacttg tagccacagc agggatgttg gagtagtctg ctcaagatac 1740	
acagaaattc gcttggtgaa tggcaagacc ccgtgtgagg gcagagtgga gctcaaaacg 1800	
cttggtgcct ggggatccct ctgtaactct cactgggaca tagaagatgc ccatgttctt 1860	
tgccagcagc ttaaatgtgg agttgccctt tctaccccag gaggagcacg ttttggaaaa 1920	
ggaaatggtc agatctggag gcatatgttt cactgcactg ggactgagca gcacatggga 1980	
gattgtcctg taactgctct aggtgcttca ttatgtcctt cagagcaagt ggcctctgta 2040	
atctgctcag gaaaccagtc ccaaacactg tcctcgtgca attcatcgtc tttgggccc 2100	
acaaggccta ccattccaga agaaagtgt gtggcctgca tagagagtgg tcaacttcgc 2160	
ctggtaaatg gaggaggtcg ctgtgctggg agagtagaga tctatcatga gggctcctgg 2220	
ggcaccatct gtgatgacag ctgggacctg agtgatgccc acgtggtttg cagacagctg 2280	
ggctgtggag aggccattaa tgccactggg tctgctcatt ttggggaagg aacaggggccc 2340	
atctggctgg atgagatgaa atgcaatgga aaagaatccc gcatttggca gtgccattca 2400	
cacggctggg ggcagcaaaa ttgcaggcac aaggaggatg cgggagttat ctgctcagaa 2460	
ttcatgtctc tgagactgac cagtgaagcc agcagagagg cctgtgcagg gcgtctggaa 2520	
gttttttaca atggagcttg gggcactggt ggcaagagta gcatgtctga aaccactgtg 2580	
ggtgtggtgt gcaggcagct gggctgtgca gacaaaggga aaatcaaccc tgcattctta 2640	
gacaaggcca tgtccattcc catgtgggtg gacaatgttc agtgtccaaa aggacctgac 2700	
acgctgtggc agtgcccatc atctccatgg gagaagagac tggccagccc ctcgaggag 2760	
acctggatca catgtgacaa caagataaga cttcaggaag gaccacttc ctgttctgga 2820	
cgtgtggaga tctggcatgg aggttcctgg gggacagtgt gtgatgactc ttgggacttg 2880	
gacgatgtc aggtgggtgt tcaacaactt ggctgtggtc cagctttgaa agcattcaaa 2940	
gaagcagagt ttggtcaggg gactggaccg atatggctca atgaagtga gtgcaaagg 3000	
aatgagtctt ccttgtggga ttgtcctgcc agacgctggg gccatagtga gtgtgggcac 3060	
aaggaagacg ctgcagtga ttgcacagat atttcagtgc agaaaacccc aaaaaagcc 3120	
acaacaggtc gctcatcccg tcagtcatcc tttattgcag tccggatcct tggggttgtt 3180	
ctgttggcca ttttcgtcgc attattcttc ttgactaaaa agcgaagaca gagacagcgg 3240	
cttgcagttt cctcaagagg agagaactta gtccaccaa ttcaataacc ggagatgaat 3300	
tcttgctga atgcagatga tctggacct atgaattcct caggaggcca ttctgagcca 3360	
cactgaaaag gaaaatggga atttataacc cagtgagttc agcctttaag ataccttgat 3420	
gaagacctgg acta 3434	
1 M S K L R M V L L E D S G S A D F R R H 1 atgagcaaactcagaatgggtgctacttgaagactctggatctgctgacttcagaagacat 21 F V N L S P F T I T V V L L L S A C F V 61 tttgtcaacctgagtccttccaccattactgtggctcttacttctcagtgcctgttttgc 41 T S S L G G T D K E L R L V D G E N K C 121 accagttctcttggaggaacagacaaggagctgaggctagtggatggtgaaaacaagtgt 61 S G R V E V K V Q E E W G T V C N N G W 181 agcgggagagtggagtgaaagtccaggaggagtggggaacggtgtgtaataatggctgg 81 S M E A V S V I C N Q L G C P T A I K A 241 agcatggaagcgggtctctgtgatttgaaccagctgggatgtccaactgctatcaaagcc 101 P G W A N S S A G S G R I W M D H V S C 301 cctggatgggctaattccagtgcaggttctggacgcatttggatggatcatgtttcttgt 121 R G N E S A L W D C K H D G W G K H S N	SEQ ID NO:18 and19

361 cgtgggaatgagtcagctctttgggattgcaaactgatggatggggaaagcatagtaac
 141 C T H Q Q D A G V T C S D G S N L E M R
 421 tgtactcaccaacaagatgctggagtgacctgctcagatggatccaatttggaaatgagg
 161 L T R G G N M C S G R I E I K F Q G R W
 481 ctgacgcgtggaggggaatatgtgttctggaagaatagagatcaaattccaaggacggtgg
 181 G T V C D D N F N I D H A S V I C R Q L
 541 ggaacagtgtgtgatgataacttcaacatagatcatgcatctgtcattttagacaactt
 201 E C G S A V S F S G S S N F G E G S G P
 601 gaatgtggaagtgtgtcagtttctctggttcatctaattttggagaaggctctggacca
 221 I W F D D L I C N G N E S A L W N C K H
 661 atctggtttgatgatcttatatgcaacggaaatgagtcagctctctggaactgcaaact
 241 Q G W G K H N C D H A E D A G V I C S K
 721 caaggatggggaaagcataactgtgacctgctgaggatgctggagtgatttgcctaaag
 261 G A D L S L R L V D G V T E C S G R L E
 781 ggagcagatctgagcctgagactggtagatggagtcactgaatgttcaggaagattagaa
 281 V R F Q G E W G T I C D D G W D S Y D A
 841 gtgagattccaaggggaatgggggacaatatgtgatgacggctgggacagttacgatgct
 301 A V A C K Q L G C P T A V T A I G R V N
 901 gctgtggcatgcaagcaactgggatgtccaactgccgtcacagccattgggtcgagttaac
 321 A S K G F G H I W L D S V S C Q G H E P
 961 gccagtaagggtttggacacatctggcttgacagcgttttcttgcaggggacatgaacct
 341 A V W Q C K H H E W G K H Y C N H N E D
 1021 gctgtctggcaatgtaaacaccatgaatggggaaagcattattgcaatcacaatgaagat
 361 A G V T C S D G S D L E L R L R G G G S
 1081 gctggcgtgacatgttctgatggatcagatctggagctaagacttagaggtggaggcagc
 381 R C A G T V E V E I Q R L L G K V C D R
 1141 cgctgtgctgggacagttgaggtggagattcagagactgttagggaagggtgtgtgacaga
 401 G W G L K E A D V V C R Q L G C G S A L
 1201 ggctggggactgaaagaagctgatgtgttgcaggcagctgggatgtggatctgcactc
 421 K T S Y Q V Y S K I Q A T N T W L F L S
 1261 aaaacatcttatcaagtgtactccaaaatccaggcaacaaacacatggctgtttctaagt
 441 S C N G N E T S L W D C K N W Q W G G L
 1321 agctgtaacggaaatgaaacttctcttgggactgcaagaactggcaatgggtggactt
 461 T C D H Y E E A K I T C S A H R E P R L
 1381 acctgtgatcactatgaagaagccaaaattacctgctcagcccacaggggaacccagactg
 481 V G G D I P C S G R V E V K H G D T W G
 1441 gttggaggggacattccctgttctggacgtgttgaagtgaagcatggtgacacgtggggc
 501 S I C D S D F S L E A A S V L C R E L Q
 1501 tccatctgtgattcggacttctctctggaagctgccagcgttctatgcagggaattacag
 521 C G T V V S I L G G A H F G E G N G Q I
 1561 tgtggcacagttgtctctatcctgggggagctcactttggagaggggaatggacagatc
 541 W A E E F Q C E G H E S H L S L C P V A
 1621 tgggctgaagaattccagtgtgagggacatgagtcctatcttctcactctgccagtagca
 561 P R P E G T C S H S R D V G V V C S R Y
 1681 ccccgccagaaggaactttagccacagcagggatgttggagtagtctgctcaagatac
 581 T E I R L V N G K T P C E G R V E L K T
 1741 acagaaattcgcttggatgaatggcaagaccccgctgtgagggcagagtggagctcaaaacg
 601 L G A W G S L C N S H W D I E D A H V L
 1801 cttggtgcctggggatccctctgtaactctcactgggacatagaagatgccatgttctt
 621 C Q Q L K C G V A L S T P G G A R F G K
 1861 tgccagcagcttaaatgtggagttgccctttctacccaggaggagcacgttttggaaaa
 641 G N G Q I W R H M F H C T G T E Q H M G
 1921 ggaaatggtcagatctggagggcatatgtttcactgcactgggactgagcagcacatggga
 661 D C P V T A L G A S L C P S E Q V A S V
 1981 gattgtcctgtaactgctctaggtgcttcattatgtccttcagagcaagtggcctctgta
 681 I C S G N Q S Q T L S S C N S S S L G P
 2041 atctgctcaggaaccagtcctccaaactgtcctcgtgcaattcatcgtctttgggcca
 701 T R P T I P E E S A V A C I E S G Q L R
 2101 acaaggcctaccattccagaagaaagtgtgtggcctgcatagagagtgggtcaacttcgc
 721 L V N G G G R C A G R V E I Y H E G S W
 2161 ctggtaaatggaggaggtcgctgtgctgggagagtagagatctatcatgagggtcctgg
 741 G T I C D D S W D L S D A H V V C R Q L
 2221 ggcaccatctgtgatgacagctgggacctgagtgatgccacgtgggttgcagacagctg
 761 G C G E A I N A T G S A H F G E G T G P
 2281 ggctgtggagaggccattaatgccactgggtctgctcattttggggaagggaacagggccc
 781 I W L D E M K C N G K E S R I W Q C H S
 2341 atctggctggatgagatgaaatgcaatggaaaagaatcccgcatgttggcagtgccattca
 801 H G W G Q Q N C R H K E D A G V I C S E
 2401 cacggctgggggcagcaaaattgcaggcacaaggaggtgctgggagttatctgctcagaa
 821 F M S L R L T S E A S R E A C A G R L E
 2461 ttcattgtctgagactgaccagtgaagccagcagagaggcctgtgcaggggcgtctggaa
 841 V F Y N G A W G T V G K S S M S E T T V
 2521 gttttttacaatggagcttggggcactgttggcaagagtagcatgtctgaaaccactgtg
 861 G V V C R Q L G C A D K G K I N P A S L
 2581 ggtgtggtgtgcaggcagctgggctgtgcagacaaagggaatcaaccctgcatcttta
 881 D K A M S I P M W V D N V Q C P K G P D
 2641 gacaaggccatgtccattcccatgtgggtggacaatgttcagtgtccaaaaggacctgac
 901 T L W Q C P S S P W E K R L A S P S E E
 2701 acgctgtggcagtgcccatcatctccatgggagaagagactggccagcccctcgaggag
 921 T W I T C D N K I R L Q E G P T S C S G
 2761 acctggatcacatgtgacaacaagataagacttcaggaaggacccacttctgttctgga
 941 R V E I W H G G S W G T V C D D S W D L
 2821 cgtgtggagatctggcatggaggttcttgggggacagtggtgtgatgactcttgggacttg

<pre> 961 D D A Q V V C Q Q L G C G P A L K A F K 2881 gacgatgctcaggtggtgtgtcaacaacttggtgtggtccagctttgaaagcattcaaa 981 E A E F G Q G T G P I W L N E V K C K G 2941 gaagcagagtttgggtcaggggactggaccgatattggtcaatgaagtgaagtgcaaaggg 1001 N E S S L W D C P A R R W G H S E C G H 3001 aatgagtccttccttgtgggattgtcctgccagacgctggggccatagtgtgtgggcac 1021 K E D A A V N C T D I S V Q K T P Q K A 3061 aaggaagacgctgcagtgaattgcacagatatttcagtgagaaaacccccacaaaaagcc 1041 T T G R S S R Q S S F I A V G I L G V V 3121 acaacaggtcgctcatcccgctcagtcattcctttattgcagtcgggatccttggggttgtt 1061 L L A I F V A L F F L T K K R R Q R Q R 3181 ctgttggccattttcgtgcattattccttcttgactaaaaagcgaagacagagacagcgg 1081 L A V S S R G E N L V H Q I Q Y R E M N 3241 cttgcagtttctcaagaggagagaacttagtccaccaaattcaataccgggagatgaat 1101 S C L N A D D L D L M N S S G G H S E P 3301 tcttgctgaatgcagatgatctggacctaattgaattcctcaggaggccattctgagcca 1121 H 3361 cac </pre>	
<pre> 1 MSKLRMVLL E DSGSADFRRH FVNLSPTTIT VVLLLSACFV TSSLGGTDKE 51 LRLVDGENKC SGRVEVKVQE EWGTVCNNGW SMEAVSVICN QLGCPTAIKA 101 PGWANSSAGS GRIWMDHVSC RGNESALWDC KHDGWGKHSN CTHQQDAGVT 151 CSDGSNLEMR LTRGGNMCSG RIEIKFQGRW GTVCDDNFNI DHASVICRQL 201 ECGSAVSFSG SSNFGEGSGP IWFDDLICNG NESALWNCKH QGWGKHNC DH 251 AEDAGVICSK GADLSLRLVD GVTECSGRLE VRFQGEWGTI CDDGWDSYDA 301 AVACKQLGCP TAVTAIGRVN ASKGFQHIWL DSVSCQGHEP AVWQCKHHEW 351 GKHYCNHNE D AGVTCSDGSD LELRLRGGS RCAGTVEVEI QRLLGKVC DR 401 GWGLKEADV V CRQLGCGSAL KTSYQVYSKI QATNTWLFLS SCNGNETSLW 451 DCKNQWGG L TCDHYEEAKI TCSAHREPR L VGGDIPCSGR VEVKHGDTWG 501 SICDSDFS L E AASVLCRELQ CGTVVSILGG AHFGEENGQI WAEFEQCEGH 551 ESHLSLCP V A PRPEGTC SHS RDVGVC SRY TEIRLVNGKT PCEGRVELKT 601 LGAWGSLC N S HWDIEDAHLV CQQLKCGVAL STPGGARFGK GNGQIWRHMF 651 HCTGT EQHMG DCPVTALGAS LCPSEQVASV ICSGNQSQT L SSCNSSSLGP 701 TRPTIPEE S A VACIESGQLR LVNGGGRCAG RVEIYHEGSW GTICDDSWDL 751 SDAHVVCR Q L GCGEAINATG SAHFGE GTGP IWLDEM KCNG KESRIWQCHS 801 HGWGQQNCR H KEDAGVICSE FMSLRLTSEA SREACAGRLE VFYNGAWGT V 851 GKSSMSET T V GVVCRLGCA DKGKINP ASL DKAMSIPMWV DNVQCPKGP D 901 TLWQCPSP W EKRLASPSEE TWITCDNKIR LQEGPTSCSG RVEIWHGGSW 951 GTVCDDSW D L DDAQVVCQQL GCGPALKAFK EAEFGQGTGP IWLNEVKCKG 1001 NESSLWD C P A RRWGHSECGH KEDAAVNCTD ISVQKTPQKA TTGRSSRQSS 1051 FIAVGILGV V LLAIFVALFF LTKKRRQRQR LAVSSRGENL VHQIQYREMN 1101 SCLNADDL D L MNSSGGHSEP H </pre>	SEQ ID NO:19

Example 7: Cloning and characterization of murine CD163

Based on the murine CD163 sequence in GenBank (AF274883), a forward primer Mus-new5' (SEQ ID NO: 20) (5'-

5 CACCGCGGCCGCCCACACGGAGCCATCAAAATCATCAA-3') and a reverse primer Mus-new3' (SEQ ID NO:21) (5'-

GGTACCGCGAACAAGCAAACCAATAGCAATATTGTTTAATTCCCTC-3') were designed using the PrimerSelect program. Restriction endonucleases sites for *NotI* and *KpnI* were included in 5' and 3' primers, respectively, to allow future cloning into other

10 expression vectors. Mouse peritoneal macrophages were harvested from mice 2 days after injecting thioglycollate medium into the peritoneal cavity. Total cellular RNA was prepared from peritoneal macrophages using the RNeasy kit. RT-PCR reactions and RT-PCR parameters were the same as described in Example 4, except the annealing

15 temperature was increased to 60°C and extension temperature increased to 72°C. The PCR product was purified on a 0.8% SeaKem agarose gel and directionally cloned into pCDNA3.1D/V5/His/TOPO according to the manufacturer's instructions. Several

clones with large inserts were identified for further analysis. A plasmid containing an insert (SEQ ID NO: 22) with a murine CD163 that encodes a protein of the same length as (1121 amino acids SEQ ID NO:24) and differs from Genbank AF274883 by only two amino acids (99.8% identity) was designated “pCDNA3.1D-murCD163v2”.

- 5 Another plasmid, “pCDNA3.1D-murCD163v3 was generated which contained an insert (SEQ ID NO: 25) containing a murine CD163 coding sequence (SEQ ID NO: 26) which encodes a protein of 1159 amino acids in length (SEQ ID NO: 27). It differs from AF274883 by only 3 amino acids within the first 1107 residues (99.7% identity), but the sequences diverge completely beginning at residue 1108. This is due to an
- 10 insertion of 82 nucleotides in the cDNA, and a concomitant shift in reading frame downstream of the insertion. As a result, murine CD163v3 contains 52 amino acids at its carboxy-terminus that are not homologous to the 14 carboxy-terminal residues of murine CD163v2. These two alternative versions of “full length” murine CD163 are most likely splice variant of the same gene, as has been described for human CD163
- 15 (Law, S.K., Micklem, K.J., Shaw, J.M., Zhang, X.P., Dong, Y., Willis, A.C. and Mason, D.Y. (1993) A new macrophage differentiation antigen which is a member of the scavenger receptor superfamily. *European Journal of Immunology* 23 (9), 2320-2325).

SEQUENCE							ID NO
							SEQ ID NO:22
gcttttgaat	gggtggacac	agaatggttc	ttcttggagg	tgctggatct	cctggttgta	60	
aaaggtttgt	ccatctaggt	ttctttgttg	tggctgtgag	ctcacttctc	agtgcctctg	120	
ctgtcactaa	cgctcctgga	gaaatgaaga	aggaactgag	actggcgggt	ggtgaaaaca	180	
actgtagtgg	gagagtggaa	cttaagatcc	atgacaagtg	gggcacagtg	tgacgtaacg	240	
gctggagcat	gaatgaagtg	tccgtggttt	gccagcagct	gggatgccca	acttctatta	300	
aagcccttgg	atgggctaac	tccagcgccg	gctctggata	tatctggatg	gacaaagttt	360	
cttgtacagg	gaatgagtca	gctctttggg	actgcaaaca	tgatgggtgg	ggaaagcata	420	
actgtaccca	tgaaaaagat	gctggagtga	cctgctcaga	tggatctaata	ttggagatga	480	
gactggtgaa	cagtgcgggc	caccgatgct	taggaagagt	agaaataaag	ttccagggaa	540	
agtgggggac	ggtgtgtgac	gacaacttca	gcaaagatca	cgcttctgtg	atttgtaaac	600	
agcttggatg	tggaagtgcc	attagtttct	ctggctcagc	taaattggga	gctggttctg	660	
gaccaatctg	gctcgatgac	ctggcatgca	atggaaatga	gtcagctctc	tgggactgca	720	
aacaccgggg	atggggcaag	cataactgtg	accatgctga	ggatgtcggg	gtgatttgct	780	
tagagggagc	agatctgagc	ctgagactag	tggatggagt	gtccagatgt	tcaggaagat	840	
tggaagtgag	attccaagga	gaatggggga	cgtgtgtga	tgataactgg	gatctccggg	900	
atgcttctgt	ggtgtgcaag	caactgggat	gtccaactgc	catcagtgcc	attggctcag	960	
ttaatgccag	tgagggatct	ggacagatct	ggcttgacaa	catttcatgc	gaaggacatg	1020	
aggcaactct	ttgggagtgt	aaacaccaag	agtggggaaa	gcattactgt	catcatagag	1080	
aagacgctgg	cgtgacatgt	tctgatggag	cagatctgga	acttagactt	gtaggtggag	1140	

gcagtcgctg tgctggcatt gtggaggtgg agattcagaa gctgactggg aagatgtgta	1200
gccgaggctg gacactggca gatgcggatg tggtttgcag acagcttgga tgtggatctg	1260
cgcttcaaac ccaggctaag atctactcta aaactggggc aacaaatacg tggctctttc	1320
ctggatcttg taatggaaat gaaactactt tttggcaatg caaaaactgg cagtggggcg	1380
gcctttcctg tgataatttc gaagaagcca aagttacctg ctgaggccac agggaaacca	1440
gactgggttg aggagaaatc ccatgctctg gtcgtgtgga agtgaaacac ggagacgtgt	1500
ggggctccgt ctgtgatttt gacttgtctc tggaagctgc cagtgtggtg tgcagggaat	1560
tacaatgtgg aacagtcgtc tctatcctag ggggagcaca ttttgagaa ggaagtggac	1620
agatctgggg tgaagaattc cagtgtagtg gggatgagtc ccatctttca ctatgctcag	1680
tggcgcccc gctagacaga acttgtacct acagcaggga tgtcagcgta gtctgctcac	1740
gatacataga tattcgtctg gcaggcggcg agtcctcctg tgagggaaga gtggagctca	1800
agacactcgg agcctggggc cccctctgca gttctcattg ggacatggaa gatgctcatg	1860
tcttatgtca gcagctgaag tgtgggggtg cccaatctat tccagaagga gcacattttg	1920
ggaaaggagc tggtcaggtc tggagtcaca tgttccactg cactggaact gaggaacata	1980
taggagattg cctcatgact gctctgggtg cgccgacgtg ttccgaagga cagggtggcct	2040
ctgtcatctg ctgaggaaac caatcccaga cactattgcc atgtagttca ttgtctccag	2100
tccaaacaac aagctctaca attccaaagg agagtgaagt tccctgcata gcaagtggcc	2160
agcttcgctt ggtaggtgga ggtggctcgt gcgctggaag agtggaggtc taccacgagg	2220
gctcttgggg caccgtctgt gatgacaatt gggatatgac tgatgccaat gtgggtgtgca	2280
agcagctgga ctgtggcgtg gcaattaacg ccactggctc tgcttacttc ggggaaggag	2340
caggagctat ctggctagac gaagtcactc gcactgggaa agagtctcat atttggcagt	2400
gccattcaca tggctgggga cgccataact gcaggcacia agaagatgca ggtgttatct	2460
gctccgagtt catgtctctg aggctgacca acgaagccca caaagaaaac tgcacaggtc	2520
gccttgaagt gttttacaat ggtacatggg gcagtattgg cagtagcaat atgtctccaa	2580
ccactgtggg ggtgggtgtg cgtcagctgg gctgtgcaga caacgggact gtgaaacca	2640
taccttcaga caagacacca tccaggccca tgtgggtaga tegtgtgcag tgtccaaaag	2700
gagttgacac tttgtggcag tgcccctcgt cacttggaa acagagacag gccagccct	2760
cctcccagga gtcttgatc atctgtgaca aaaaaataag actccaggaa gggcatacag	2820
actgttctgg acgtgtggag atctggcaca aaggttcctg gggaaacagtg tgtgatgact	2880
cctgggatct taatgatgct aaggttgtat gtaagcagtt gggctgtggc caagctgtga	2940
aggcaactaaa agaagcagca tttgggtccag gaactgggac catatggctc aatgaaatta	3000
agtgtagagg gaatgagtc tccctgtggg attgtcctgc caaacgtgg agtcacagcg	3060
actgtgggca caaagaagat gcttccatcc agtgccctcc aaaaatgact tcagaatcac	3120
atcatggcac aggtcacccc accctcacgg cactcttggg ttgtggagcc attctattgg	3180
tcctcctcat tgtcttcctc ctgtggactc tgaagcgacg acagattcag cgacttacag	3240
tttcctcaag aggagaggtc ttgatacatc aagttcagta ccaagagatg gattcaaagg	3300
cggatgatct ggacttgctg aaatcctcgg gggtcattca gaggcacact gagaaggaaa	3360
atgataatct ataatccact gaggttggag ttttaagaagc cttgacagga cagccagcta	3420
aatggaacaa gagcccaggc aacgcacgga tgaccacagc tgcattctca tgcagtcctt	3480
tgtttcctgg aactctgctg aacctgcaaa aaccatattt gtgaatgtga ccacttaata	3540
gagatgggag actttt	3556

1 M G G H R M V L L G G A G S P G C K R F 1 atgggtggacacagaatggttcttcttggaggtgctggatctcctggttgtaaaaggttt 21 V H L G F F V V A V S S L L S A S A V T 61 gtccatctaggtttcttcttgttggctgtgagctcacttctcagtgctctgtgtcact 41 N A P G E M K K E L R L A G G E N N C S 121 aacgctcctggagaaatgaagaaggaactgagactggcgggtggtgaaaacaactgtagt 61 G R V E L K I H D K W G T V C S N G W S 181 gggagagtggaaacttaagatccatgacaagtggggcacagtgtgcagtaacggctggagc 81 M N E V S V V C Q Q L G C P T S I K A L 241 atgaatgaagtgtccgtggtttgcccagcagctgggatgcccacttctattaaagccctt 101 G W A N S S A G S G Y I W M D K V S C T 301 ggatgggctaactccagcggcgtctggatatactggatggacaaagtcttctgtaca 121 G N E S A L W D C K H D G W G K H N C T 361 gggaatgagtcagctcttgggactgcaaacatgatgggtggggaagcataactgtacc 141 H E K D A G V T C S D G S N L E M R L V 421 catgaaaaagatgctggagtgcctgctcagatggatctaatttggagatgagactgggtg 161 N S A G H R C L G R V E I K F Q G K W G 481 aacagtgcgggccaccgatgcttaggaagagtagaaataaagttccagggaagtggggg 181 T V C D D N F S K D H A S V I C K Q L G 541 acggtgtgtgacgacaacttcagcaaagatcacgcttctgtgatttgtaaacagcttgga 201 C G S A I S F S G S A K L G A G S G P I 601 tgtggaagtgccattagtttctctggctcagctaaattgggagctggttctggaccaatc 221 W L D D L A C N G N E S A L W D C K H R 661 tggctcgatgacctggcatgcaatggaaatgagtcagctctctgggactgcaaaccaccg 241 G W G K H N C D H A E D V G V I C L E G 721 ggatggggcaagcataactgtgacctgctgaggatgtcgggtgtgatttgcttagaggga 261 A D L S L R L V D G V S R C S G R L E V 781 gcagatctgagcctgagactagtggatggatgtccagatgttcaggaagattggaagtg 281 R F Q G E W G T V C D D N W D L R D A S 841 agattccaaggagaatgggggaccgtgtgtgatgataactgggatctccgggatgcttct 301 V V C K Q L G C P T A I S A I G R V N A 901 gtggtgtgcaagcaactgggatgtccaactgccatcagtgccattgggtcgagttaatgcc 321 S E G S G Q I W L D N I S C E G H E A T 961 agtgagggatctggacagatttggcttgacaacatttcatgcgaaggacatgaggcaact 341 L W E C K H Q E W G K H Y C H H R E D A 1021 ctttgggagtgtaaacaccaagagtggggaaagcattactgtcatcatagagaagacgct 361 G V T C S D G A D L E L R L V G G G S R 1081 ggcgtgacatgttctgatggagcagatctggaacttagactttaggtggaggcagtcgc 381 C A G I V E V E I Q K L T G K M C S R G 1141 tgtgctggcattgtggaggtggagattcagaagctgactgggaagatgtgtagccgaggc 401 W T L A D A D V V C R Q L G C G S A L Q 1201 tggacactggcagatgcggatgtggttgcagacagcttggatgtggatctgcgcttcaa 421 T Q A K I Y S K T G A T N T W L F P G S 1261 acccaggctaagatctactctaaaactggggcaacaaatacgtggctctttcctggatct 441 C N G N E T T F W Q C K N W Q W G G L S 1321 tgtaatggaaatgaaactacttttggcaactgcaaaaactggcagtggggcccctttcc 461 C D N F E E A K V T C S G H R E P R L V 1381 tgtgataatttcgaagaagccaaagtacctgctcaggccacagggaacccagactggtt 481 G G E I P C S G R V E V K H G D V W G S 1441 ggaggagaaatcccatgctctggctggtggaagtgaacacggagacgtgtggggctcc 501 V C D F D L S L E A A S V V C R E L Q C 1501 gtctgtgattttgacttgtctctggaagctgccagtgtggtgtgcagggaattacaatgt 521 G T V V S I L G G A H F G E G S G Q I W 1561 ggaacagtcgtctctatcctagggggagcacattttggagaaggaagtggacagatctgg 541 G E E F Q C S G D E S H L S L C S V A P 1621 ggtgaagaattccagtgtagtggggatgagtccttctcactatgctcagtgggcgccc 561 P L D R T C T H S R D V S V V C S R Y I 1681 ccgctagacagaacttgtacccacagcaggatgtcagcgtagtctgctcacgatacata 581 D I R L A G G E S S C E G R V E L K T L 1741 gatattcgtctggcaggcggcgagtcctcctgtgaggggaagagtggagctcaagacactc 601 G A W G P L C S S H W D M E D A H V L C 1801 ggagcctggggctccctctgcagttctcattgggacatggaagatgctcatgtcttatgt 621 Q Q L K C G V A Q S I P E G A H F G K G 1861 cagcagctgaagtgtggggttggccaatctattccagaaggagcacattttgggaaagga 641 A G Q V W S H M F H C T G T E E H I G D 1921 gctgggtcaggtctggagtcacatgttccactgcactggaactgaggaacatataggagat 661 C L M T A L G A P T C S E G Q V A S V I 1981 tgctcatgactgctctgggtgcgcccagctgttccgaaggacaggtggcctctgtcatc 681 C S G N Q S Q T L L P C S S L S P V Q T 2041 tgctcaggaaaccaatcccagacactattgccatgtagttcattgtctccagtcctaaaca 701 T S S T I P K E S E V P C I A S G Q L R 2101 acaagctctacaattccaaaggagagtgaagttccctgcatagcaagtgccagcttcgc 721 L V G G G G R C A G R V E V Y H E G S W 2161 ttggtaggtggaggtggtcgctgcgctggaagagtggaggtctaccacgagggtcttgg 741 G T V C D D N W D M T D A N V V C K Q L 2221 ggcaccgtctgtgatgacaattgggatatgactgatgccaatgtggtgtgcaagcagctg 761 D C G V A I N A T G S A Y F G E G A G A	SEQ ID NO:23and 24

2281 gactgtggcgtggcaattaacgccactggctctgcttacttcggggaaggagcaggagct 781 I W L D E V I C T G K E S H I W Q C H S 2341 atctggctagacgaagtcatctgcactgggaaagagtctcatatttggcagtgccattca 801 H G W G R H N C R H K E D A G V I C S E 2401 catggctggggacgccataactgcagggcacaagaagatgcaggtgttatctgctccgag 821 F M S L R L T N E A H K E N C T G R L E 2461 ttcatgtctctgaggctgaccaacgaagcccacaaagaaaactgcacaggtcgcttgaa 841 V F Y N G T W G S I G S S N M S P T T V 2521 gtgttttacaatggtacatggggcagtattggcagtagcaatatgtctccaaccactgtg 861 G V V C R Q L G C A D N G T V K P I P S 2581 ggggtgtgtgcccgtcagctgggctgtgcagacaacgggactgtgaaacccataccttca 881 D K T P S R P M W V D R V Q C P K G V D 2641 gacaagacaccatccaggcccatgtgggttagatcgtgtgcagtgtccaaaaggagttgac 901 T L W Q C P S S P W K Q R Q A S P S S Q 2701 actttgtggcagtggccctcgtcaccttggaaacagagacaggccagccctcctcccag 921 E S W I I C D N K I R L Q E G H T D C S 2761 gagtcttgatcatctgtgacaacaaaataagactccaggaagggcatacagactgttct 941 G R V E I W H K G S W G T V C D D S W D 2821 ggacgtgtggagatctggcacaaagggttctggggaacagtgtgtgatgactcctgggat 961 L N D A K V V C K Q L G C G Q A V K A L 2881 cttaatgatgctaagggttgatgtaagcagttgggctgtggccaagctgtgaaggcacta 981 K E A A F G P G T G P I W L N E I K C R 2941 aaagaagcagcatttgggtccaggaactgggcccataatggctcaatgaaattaagtgtaga 1001 G N E S S L W D C P A K P W S H S D C G 3001 gggaatgagtcttccctgtgggattgtcctgccaacccgtggagtcacagcgactgtggg 1021 H K E D A S I Q C L P K M T S E S H H G 3061 cacaagaagatgcttccatccagtgcctcccaaaatgacttcagaatcacatcatggc 1041 T G H P T L T A L L V C G A I L L V L L 3121 acaggtcacccaccctcacggcactccttggtttgtggagccattctattggtcctcctc 1061 I V F L L W T L K R R Q I Q R L T V S S 3181 attgtcttctcctgtggactctgaagcgacgacagattcagcgacttacagtttctcctca 1081 R G E V L I H Q V Q Y Q E M D S K A D D 3241 agaggagaggtcttgatacatcaagttcagtagcaagagatggattcaaaggcggatgat 1101 L D L L K S S G V I Q R H T E K E N D N 3301 ctggacttgctgaaatcctcgggggtcattcagaggcacactgagaaggaaaatgataat 1121 L 3361 tta						
1 51 101 151 201 251 301 351 401 451 501 551 601 651 701 751 801 851 901 951 1001 1051 1101	MGGHRMVLG RLAGGENNCS GWANSSAGSG DGSNLEMLV CGSAISFSGS EDVGVICLEG VVCKQLGCPT KHYCHHREDA WTLADADVVC CKNWQWGGLS VCDFDLSLEA SHLSLCSVAP GAWGPLCSSH CTGTEEHIGD TSSTIPKESE TDANVCKQL HGWGRHNCRH GSSNMSPTTV TLWQCPSSPW WGTVCDDSWD GNESLWDCCP VCGAILLVLL LDLLKSSGVI	GAGSPGCKRF GRVELKIHDK YIWMKVSC NSAGHRCLGR AKLGAGSGPI ADLSLRLVDG AISAIGRVNA GVTCSDGADL RQLGCGSALQ CDNFEEAKVT ASVVCRELQC PLDRTCTHSR WDMEDAHVLC CLMTALGAPT VPCIASGQLR DCGVAINATG KEDAGVICSE GVVCRQLGCA KQRQASPSSQ LNDKVVCKQ AKPWSHSDCG IVFLLWTLKR QRHTEKENDN	VHLGFFVAV WGTVCNSGWS GNESALWDCK VEIKFQKKG WLDDLACNGN VSRCSGRLEV SESGQIWL ELRLVGGGSR TQAKIYSKTG CSGHREPRLV GTVVSVILGA DVSVVCSRYI QQLKCGVAQS CSEGQVASVI LVGGGRCAG FMSLRLTNEA DNGTVKPIPS DKTPSRPMWV ESWIICDNKI LGCGQAVKAL HKEDASIQCL RQIQRLTVSS L	SSLLSASAVT MNEVSVVCQQ HDGWGKHNT TVCCDNFSKD ESALWDCKHR RFQGEWGTVC NISCEGHEAT CAGIVEVEIQ ATNTWLFPGS GGEIPCSGRV HFGEQSGQIW DIRLAGGESS IPEGAHFGKG CSGNQSQTLL RVEVYHEGSW IWLDEVICTG HKENCTGRLE DKTPSRPMWV RLQEGHTDCS KEAAGFGPTG PKMTSESHHG RGEVLIHQVQ	NAPGEMKKEL LGCPTSIKAL HEKDAGVTC HASVICKQLG GWGKHNCDA DDNWDLRDAS LWECKHQEWG KLTGKMCSR CNGNETTFWQ EVKHGDVWGS GEEFQCSGDE CEGRVELKTL AGQVWSHMFH PCSSLSPVQT GTVCDDNWD KESHIWQCHS VFYNGTWGSI DRVQCCKGVD GRVEIWHKGS PIWLNEIKCR TGHPTLTALL YQEMDSKADD	SEQ ID NO:24
gctttggaat ggggtggacac agaatgggttc ttcttggagg tgctggatct cctggttgta 60 aaaggtttgt ccatctaggt ttctttgttg tggctgtgag ctcacttctc agtgcctctg 120 ctgtcactaa cgctcctgga gaaatgaaga aggaactgag actggcgggt ggtgaaaaca 180 actgtagtgg gagagtggaa cttaagatcc atgacaagtg gggcacagtg tgcagtaacg 240 gctggagcat gaatgaagtg tccgtgggtt gccagcagct gggatgcca acttctatta 300 aagcccttgg atgggctaac tccagcgccg gctctggata tatctggatg gacaaagttt 360 cttgtagagg gaatgagtca gctctttggg actgcaaaca tgatgggtgg ggaaagcata 420 actgtacca tgaaaaagat gctggagtga cctgctcaga tggatctaatt ttggagatga 480 gactggtgaa cagtgcgggc caccgatgct taggaagagt agaaataaag ttccaggga 540						ID NO:25

agtgggggac ggtgtgtgac gacaacttca gcaaagatca cgcttctgtg atttgtaaac	600	
agcttggatg tggaagtgcc attagtttct ctggctcagc taaattggga gctggttctg	660	
gaccaatctg gctcgatgac ctggcatgca atggaaatga gtcagctctc tgggactgca	720	
aacaccgggg atggggcaag cataactgtg accatgctga ggatgtcggt gtgatttgct	780	
tagaggggagc agatctgagc ctgagactag tggatggagt gtccagatgt tcaggaagat	840	
tggaagtgag attccaagga gaatggggga ccgtgtgtga tgataactgg gatctccggg	900	
atgcttctgt ggtgtgcaag caactgggat gtccaactgc catcagtgcc attggctcag	960	
ttaatgccag tgagggatct ggacagattt ggcttgacaa catttcatgc gaaggacatg	1020	
aggcaactct ttgggagtgt aaacaccaag agtggggaaa gcattactgt catcatagag	1080	
aagacgctgg cgtgacatgt tctgatggag cagatctgga acttagactt gtaggtggag	1140	
gcagtcgctg tgctggcatt gtggaggtgg agattcagaa gctgactggg aagatgtgta	1200	
gccgaggctg gacactggca gatgcggatg tggtttgacg acagcttggga tgtggatctg	1260	
cgcttcaaac ccaggctaag atctactcta aaactggggc aacaaatacg tggctctttc	1320	
ctggatcttg taatggaaat gaaactactt tttggcaatg caaaaactgg cagtggggcg	1380	
gcctttcctg tgataatttc gaagaagcca aagttacctg ctccaggccac agggaaacca	1440	
gactggttgg aggagaaatc ccatgctctg gtcgtgtgga aatgaaacac ggagacgtgt	1500	
ggggctccgt ctgtgatttt gacttgtctc tggaaactgc cagtgtggtg tgcagggaat	1560	
tacaatgtgg aacagtcgtc tctatcctag ggggagcaca ttttgagaa ggaagtggac	1620	
agatctgggg tgaagaattc cagtgtagtg gggatgagtc ccatctttca ctatgctcag	1680	
tggcgcccc gctagacaga acttgtacct acagcaggga tgtcagcgta gtctgtcac	1740	
gatacataga tttcgtctg gcaggcggcg agtcctcctg tgagggaaga gtggagctca	1800	
agacactcgg agcctggggg cccctctgca gttctcattg ggacatggaa gatgctcatg	1860	
tcttatgtca gcagctgaag tgtgggggtg cccaatctat tccagaagga gcacattttg	1920	
ggaaaggagc tggtcaggtc tggagtcaca tgttccactg cactggaact gaggaacata	1980	
taggagattg cctcatgact gctctgggtg cgccgacgtg ttccgaagga cagggtggct	2040	
ctgtcatctg ctccaggaaac caatcccaga cactattgcc atgtagtcca ttgtctccag	2100	
tccaaacaac aagctctaca attccaaagg agagtgaagt tccctgcata gcaagtggcc	2160	
agcttcgctt ggtaggtgga ggtggctcgt gcgctggaag agtggaggtc taccacgagg	2220	
gctcttgggg caccgtctgt gatgacaatt gggatatgac tgatgccaat gtggtgtgca	2280	
agcagctgga ctgtggcgtg gcaattaacg ccactggctc tgcttacttc ggggaaggag	2340	
caggagctat ctggctagac gaagtcattc gcactgggaa agagtctcat atttggcagt	2400	
gccattcaca tggctgggga cgccataact gcaggcacia agaagatgca ggtgttatct	2460	
gctccgagtt catgtctctg aggctgacca acgaagccca caaagaaaac tgcacaggtc	2520	
gccttgaagt gttttacaat ggtacatggg gcagtattgg cagtagcaat atgtctccaa	2580	
ccactgtggg ggtggtgtgc cgtcagctgg gctgtgcaga caacgggact gtgaaacca	2640	
taccttcaga caagacacca tccaggccca tgtgggtaga tctgtgtcag tgtccaaaag	2700	
gagttgacac tttgtggcag tgccctcgt caccttggaa acagagacag gccagcccct	2760	
cctcccagga gtccctggatc atctgtgaca acaaaataag actccaggaa gggcatacag	2820	
actgttctgg acgtgtggag atctggcaca aaggttctct gggaaacagt tgtgatgact	2880	
cctgggatct taatgatgct aaggttgatg gtaagcagtt gggctgtggc caagctgtga	2940	
aggcactaaa agaagcagca tttgggtccag gaactgggac catatggctc aatgaaatta	3000	
agtgtagagg gaatgagtct tccctgtggg attgtcctgc caaacctggg agtcacagcg	3060	

actgtgggca caaagaagat gcttccatcc agtgccctccc caaaatgact tcagaatcac 3120 atcatggcac aggtcacccc accctcacgg cactcttggg ttgtggagcc attctattgg 3180 tcctcctcat tgtcttcctc ctgtggactc tgaagcgacg acagattcag cgacttacag 3240 tttctcaag aggagaggtc ttgatacatc aagttcagta ccaagagatg gattcaaagg 3300 eggatgatct ggacttgctg aaatcctcgg aaaattccaa caattcatat gattttaatg 3360 atgatggact gacatctttg tctaaatata ttcctatttc tggaattaaa aaggggtcat 3420 tcagaggcac actgagaagg aaaatgataa ttataatcc actgaggttg gagtttaaga 3480 agccttgaca ggacagccag ctaaattggaa caagagccca ggcaacgcac ggatgaccac 3540 agctgcatct tcatgcagtc ctttgtttcc tggaactctg ctgaacctgc aaaaaccata 3600 tttgtgaatg tgaccactta atagagatgg gagactttt 3639	
1 M G G H R M V L L G G A G S P G C K R F 1 atgggtggacacagaatgggttcttcttgagggtgctggatctcctgggttgtaaaagggttt 21 V H L G F F V V A V S S L L S A S A V T 61 gtccatctaggtttcttcttggttggtgagctcacttctcagtgccctctgctgtcact 41 N A P G E M K K E L R L A G G E N N C S 121 aacgctcctggagaaatgaagaaggaactgagactggcgggtggtgaaaacaactgtagt 61 G R V E L K I H D K W G T V C S N G W S 181 gggagagtggaaacttaagatccatgacaagtggggcacagtgtgcagtaacggctggagc 81 M N E V S V V C Q Q L G C P T S I K A L 241 atgaatgaagtgtccgtgggttgccagcagctgggatgcccaacttctattaaagccctt 101 G W A N S S A G S G Y I W M D K V S C T 301 ggatgggctaactccagcgcggctctggatatctggatggacaaagtttcttgtaca 121 G N E S A L W D C K H D G W G K H N C T 361 gggaatgagtcagctcttgggactgcaaacatgatgggtggggaagcataactgtacc 141 H E K D A G V T C S D G S N L E M R L V 421 catgaaaaagatgctggagtgcctgctcagatggatctaatttggagatgagactggtg 161 N S A G H R C L G R V E I K F Q G K W G 481 aacagtgcgggcccacgatgcttaggaagagttagaaataaagttccagggaagtggtggg 181 T V C D D N F S K D H A S V I C K Q L G 541 acggtgtgtgacgacaacttcagcaaatgacgcttctgtgatttgaacagcttga 201 C G S A I S F S G S A K L G A G S G P I 601 tgtggaagtgccattagtttctctggctcagctaaattgggagctggttctggaccaatc 221 W L D D L A C N G N E S A L W D C K H R 661 tggctcgatgacctggcatgcaatggaaatgagtcagctctctgggactgcaaacaccgg 241 G W G K H N C D H A E D V G V I C L E G 721 ggatggggcaagcataactgtgaccatgctgaggatgtcggtgtgatttgccttagaggga 261 A D L S L R L V D G V S R C S G R L E V 781 gcagatctgagcctgagactagtggatggagtgtccagatgttcaggaagattggaagtg 281 R F Q G E W G T V C D D N W D L R D A S 841 agattccaaggagaatgggggaccgtgtgtgatgataactgggatctccgggatgcttct 301 V V C K Q L G C P T A I S A I G R V N A 901 gtggtgtgcaagcaactgggatgtccaactgccatcagtgccattggtcgagttaatgcc 321 S E G S G Q I W L D N I S C E G H E A T 961 agtgaggatctggacagatttggcttgacaacatttcatgcgaaggacatgaggcaact 341 L W E C K H Q E W G K H Y C H H R E D A 1021 ctttgggagtgtaaacaccaagagtggggaaagcattactgtcatcatagagaagacgct 361 G V T C S D G A D L E L R L V G G G S R 1081 ggcgtgacatgttctgatggagcagatctggaacttagactttaggtggaggcagtcgc 381 C A G I V E V E I Q K L T G K M C S R G 1141 tgtgctggcattgtggaggtggagattcagaagctgactgggaagatgtgtagccgaggc 401 W T L A D A D V V C R Q L G C G S A L Q 1201 tggacactggcagatgcggatgtggttgcagacagcttggatgtggatctgcgcttcaa 421 T Q A K I Y S K T G A T N T W L F P G S 1261 acccaggctaagatctactctaaaactggggcaacaaatacgtggctcttctcctggatct 441 C N G N E T T F W Q C K N W Q W G G L S 1321 tgtaatggaaatgaaactacttttggcaatgcaaaaactggcagtgggcgcccttcc 461 C D N F E E A K V T C S G H R E P R L V 1381 tgtgataatttcgaagaagccaaagttacctgctcaggccacaggggaaccagactggtt 481 G G E I P C S G R V E M K H G D V W G S 1441 ggaggagaaatcccatgctctggtcgtgtggaaatgaaacacggagacgtgtggggtcc 501 V C D F D L S L E A A S V V C R E L Q C 1501 gtctgtgattttagcttgtctctggaagctgccagtgtggtgtgcagggaattacaatgt 521 G T V V S I L G G A H F G E G S G Q I W 1561 ggaacagtcgtctctatcctagggggagcacatttggagaaggaagtggacagatctgg 541 G E E F Q C S G D E S H L S L C S V A P 1621 ggtgaagaattccagtgtagtggggatgagtcacatcttctactatgctcagtgggcgc 561 P L D R T C T H S R D V S V V C S R Y I 1681 ccgctagacagaacttgtacccacagcagggatgtcagcgtagtctgctcacgatacata 581 D I R L A G G E S S C E G R V E L K T L 1741 gatattcgtctggcaggcgaggctcctcctgtgaggggaagagtggagctcaagacactc	ID NO:26 and 27

601	G A W G P L C S S H W D M E D A H V L C	
1801	ggagcctgggggtccctctgcagttctcattgggacatggaagatgctcatgtcttatgt	
621	Q Q L K C G V A Q S I P E G A H F G K G	
1861	cagcagctgaagtgtggggttgcccaatctattccagaaggagcacattttgggaaagga	
641	A G Q V W S H M F H C T G T E E H I G D	
1921	gctgggtcaggtctggagtcacatgttccactgcactggaactgaggaacatataggagat	
661	C L M T A L G A P T C S E G Q V A S V I	
1981	tgcctcatgactgctctgggtgcccgcagctgttccgaaggacaggtggcctctgtcatc	
681	C S G N Q S Q T L L P C S S L S P V Q T	
2041	tgctcaggaaccaatcccagacactattgccaatgtagttcattgtctccagtccaaaca	
701	T S S T I P K E S E V P C I A S G Q L R	
2101	acaagctctacaattccaaaggagagtgaagttccctgcatagcaagtggccagcttcgc	
721	L V G G G G R C A G R V E V Y H E G S W	
2161	ttggtaggtggaggtggctcgctgcgctggaagagtggaggtctaccacgagggctcttgg	
741	G T V C D D N W D M T D A N V V C K Q L	
2221	ggcaccgtctgtgatgacaattgggatatgactgatgccaatgtggtgtgcaagcagctg	
761	D C G V A I N A T G S A Y F G E G A G A	
2281	gactgtggcgtggcaattaacgccactggctctgcttacttcggggaaggagcaggagct	
781	I W L D E V I C T G K E S H I W Q C H S	
2341	atctggctagacgaagtcatctgcactgggaaagagtctcatatttggcagtgccattca	
801	H G W G R H N C R H K E D A G V I C S E	
2401	catggctggggacgccataactgcaggcacaaagaagatgcaggtgttatctgtccgag	
821	F M S L R L T N E A H K E N C T G R L E	
2461	ttcatgtctctgaggctgaccaagcgaagcccacaaagaaaactgcacaggtcgccttgaa	
841	V F Y N G T W G S I G S S N M S P T T V	
2521	gtgtttttacaatgggtacatggggcagtagttggcagtagcaatatgtctccaaccactgtg	
861	G V V C R Q L G C A D N G T V K P I P S	
2581	ggggtgggtgtgccgtcagctgggctgtgcagacaacgggactgtgaaaccataccttca	
881	D K T P S R P M W V D R V Q C P K G V D	
2641	gacaagacaccatccaggcccatgtgggtagatcggtgtgcagtgtccaaaaggagttgac	
901	T L W Q C P S S P W K Q R Q A S P S S Q	
2701	actttgtggcagtggccctcgctcaccttggaacagagacaggccagccctcctcccag	
921	E S W I I C D N K I R L Q E G H T D C S	
2761	gagtcctggatcatctgtgacaacaaaataagactccaggaagggcatacagactgttct	
941	G R V E I W H K G S W G T V C D D S W D	
2821	ggacgtgtggagatctggcacaaagggttcctggggaacagtgtgtgatgactcctgggat	
961	L N D A K V V C K Q L G C G Q A V K A L	
2881	cttaatgatgctaagggttgatgtaagcagttgggctgtggccaagctgtgaaggcacta	
981	K E A A F G P G T G P I W L N E I K C R	
2941	aaagaagcagcatttgggtccaggaactgggcccataatggctcaatgaaattaagtgtaga	
1001	G N E S S L W D C P A K P W S H S D C G	
3001	gggaatgagtccttcctgtgggattgtcctgcccacacagctggagtcacagcgactgtggg	
1021	H K E D A S I Q C L P K M T S E S H H G	
3061	cacaaagaagatgcttccatccagtgcctcccaaatgacttcagaatcacatcatggc	
1041	T G H P T L T A L L V C G A I L L V L L	
3121	acaggtcaccaccctcacggcactcttgggttgggagccattctattggtcctcctc	
1061	I V F L L W T L K R R Q I Q R L T V S S	
3181	attgtcttctcctgtggactctgaagcgacgacagattcagcgacttacagtttctca	
1081	R G E V L I H Q V Q Y Q E M D S K A D D	
3241	agaggagaggtcttgatacatcaagttcagtagcaagagatggattcaaaggcgatgat	
1101	L D L L K S S E N S N N S Y D F N D D G	
3301	ctggacttgctgaaatcctcggaaaattccaacaattcatatgattttaatgatgatgga	
1121	L T S L S K Y L P I S G I K K G S F R G	
3361	ctgacatctttgtctaaatatcttctatttctggaattaaaaaggggtcattcagaggc	
1141	T L R R K M I I Y N P L R L E F K K P	
3421	acactgagaaggaaaatgataatttataatccactgaggttggagtttaagaagcct	
1	MGGHRMVLIG GAGSPGCKRF VHLGFFVAV SSLLSASAVT NAPGEMKKEL	ID NO:27
51	RLAGGENNCS GRVELKIHDK WGTVCNSGWS MNEVSVVCQQ LGCPTSIKAL	
101	GWANSSAGSG YIWMKVSC T GNESALWDCK HDGWGKHNCT HEKDAGVTC	
151	DGSNLEMRLV NSAGHRC LGR VEIKFQKKG TVCDDNFSKD HASVICKQLG	
201	CGSAISFSGS AKLGAGSGPI WLDDLACNGN ESALWDCKHR GWGKHNCDA	
251	EDVGVICLEG ADLSRLVDG VSRCSGRLEV RFQGEWGTVC DDNWDLRDAS	
301	VVCKQLGCPT AISAIGRNA SEGSQIWL NISCEGHEAT LWECKHQEWG	
351	KHYCHHREDA GVTCSGDADL ELRLVGGGSR CAGIVEVEIQ KLTGKMCSR	
401	WTLADADVVC RQLGCGSALQ TQAKIYSKTG ATNTWLFPGS CNGNETTFWQ	
451	CKNWQWGLS CNDFEEAKVT CSGHREPLV GGEIPCSGRV EMKHGDVWGS	
501	VCDLFLSLEA ASVVCRELQC GTVVSILGGA HFGEQSGQIW GEEFQCSGDE	
551	SHLSLCSVAP PLDRCTHSR DVSVCSTRYI DIRLAGGESS CEGRVELKTL	
601	GAWGPLCSSH WDMEDAHVLC QQLKCGVAQS IPEGAHFGKG AGQVWSHMFH	
651	CTGTEEHIGD CLMTALGAPT CSEGQVASVI CSGNQSQTL PCSSLSFVQT	
701	TSSTIPKESE VPCIASGQLR LVGGGRCAG RVEVYHEGSW GTVCDNDWDM	
751	TDANVVCKQL DCGVAINATG SAYFGEGAGA IWLDEVICTG KESHIWQCHS	
801	HGWGRHNCRH KEDAGVICSE FMSLRILTNEA HKENCTGRLE VFYNGTWGSI	
851	GSSNMSPTTV GVVCRLGCA DNGTVKPIPS DKTPSRPMWV DRVQCPKGV	
901	TLWQCPSSPW KQRQASPSQ ESWICDNKI RLQEGHTDCS GRVEIWHKGS	
951	WGTVCDDSWD LNDKVVCKQ LGCGQAVKAL KEAAGFGPGT PIWLNEIKCR	
1001	GNESSLWDCP AKPWSHSDCG HKEDASIQCL PKMTSESHHG TGHPTLTALL	
1051	VCGAILLVLL IVFLLWTLKR RQIQRLTVSS RGEVLIHQVQ YQEMDSKADD	
1101	LDLLKSSENS NNSYDFNDG LTSLSKYLP I SGIKKGSRG TLRRKMIIYN	
1151	PLRLEFKKP	

Example 8: Cloning and characterization of MARC-145 CD163

A forward primer 5'simianCD163 (SEQ ID NO: 28) (5'-CACCGGAATGAGCAAACCTCAGAATGG-3' based on human CD163) and a reverse primer HuCD163-3'Kpn (SEQ ID NO:29) (5'-TGCTCCGGTACCTAGTCCAGGTCTTCATCAAGGTATCTTA-3') were used to amplify CD163 cDNA from MARC-145 African Green Monkey kidney cells. Total cellular RNA was prepared from MARC-145 cells using the RNeasy kit. RT-PCR parameters were the same as described in Example 4. RT-PCR products were cloned directionally into the pCDNA3.1D/V5/His/TOPO vector according to the manufacturer's instruction. Several clones containing large inserts were analyzed. Clone #25 was designated "pCDNA3.1D-MARC-CD163v2". This novel CD163 cDNA from MARC-145 cells is 1116 amino acids in length. When compared to the sequences in GenBank database, the MARC-145 CD163 amino acid sequence is 96.3% identical to human CD163 (Genbank Z22968), 84.7% identical to pig CD163 (Genbank AJ311716), and 73.9% identical to mouse CD163 (Genbank AF274883).

SEQUENCE							ID NO
atgagcaaac	tcagaatggt	gctacttgaa	gactctggat	ctgctgacgt	cagaagacat	60	SEQ ID NO: 30
tttgtcaact	tgagtcacct	cactattgct	gtggctcttac	ttctccgtgc	ctgttttgtc	120	
accagttctc	ttggaggaac	aaccaaggag	ctgaggctag	tggatggtga	aaacaagtgt	180	
agtgggagag	tggaagtga	aatccaggag	gagtggggaa	cgggtgtgtaa	taatggctgg	240	
agcatggaag	cagtctctgt	gatttgtaac	cagctgggat	gtccaactgc	tatcaaagcc	300	
actggatggg	ctaattccag	tgcaggttct	ggacgcattt	ggatggatca	tgtttcttgt	360	
cgtgggaatg	agtcagctct	ttgggactgc	aaacatgatg	gatggggaaa	gcatagtaac	420	
tgtactcacc	aacaagatgc	tggagtgact	tgtcagatg	gatccgattt	ggaaatgagg	480	
ctgacgaatg	gagggaatat	gtgttctgga	agaatagaga	tcaaattcca	aggacagtgg	540	
ggaacagtgt	gtgatgataa	cttcaacatc	aatcatgcat	ctgtggtttg	taaacaactt	600	
gaatgtggaa	gtgctgtcag	tttctctggt	tcagctaatt	ttggagaagg	ctctggacca	660	
atctggtttg	atgatcttat	atgcaacgga	aatgagtcag	ctctctggaa	ctgcaaacat	720	
caaggatggg	gaaagcataa	ctgtgatcat	gctgaggatg	ctggagtgat	ttgctcaaag	780	
ggagcagatc	tgagcctgag	actggttagat	ggagtcactg	aatgttcagg	aagattagaa	840	
gtgagattcc	aaggagaatg	ggggacaata	tgtgatgacg	gctgggacag	tcatgatgct	900	
gctgtggcat	gcaagcaact	gggatgtcca	actgctatca	ccgccattgg	tcgagttaac	960	
gccagtgagg	gatttggaca	catctggcct	gacagtgttt	cttgccaggg	acatgaacct	1020	
gcggtctggc	aatgtaaaca	ccatgaatgg	ggaaagcatt	attgcaatca	caatgaagat	1080	
gctggcgtaa	catgttctga	tggatcagat	ctggagctaa	gacttagagg	tggaggcagc	1140	
cgtgtgtctg	ggacagttga	ggtggagatt	cagagactgt	tagggaaggt	gtgtgacaga	1200	
ggctggggac	tgaaagaagc	tgatgtgggt	tgcaggcagc	tgggatgtgg	atctgcactc	1260	

aaaacatcct atcaagtata ctccaaaatc caggcaacaa acatgtggct gtttctaagt 1320	
agctgtaacg gaaatgaaac ttctctttgg gactgcaaga actggcaatg gggaggactt 1380	
acctgtgate actatgaaga agccaaaatt acctgtcag cccacagga acccagactg 1440	
gttggaggag acattccctg ttctggacgc gttgaagtga agcatggtga cacatggggc 1500	
tccgtctgtg attcggattt ctctctggaa gctgccagcg ttctatgcag ggaattacag 1560	
tgtggcacag tcgtctctat cctgggggga gctcactttg gagagggaaa tggacagatc 1620	
tgggctgaag aattccagtg tgagggacat gagtcccatc ttctactctg cccagtagca 1680	
ccccgccag aaggaaactg tagccacagc agggatgttg gagtagtctg ctcaagatac 1740	
acagaaattc gcttgggtgaa tggcaagacc ccatgtgagg gcagagtgga gctcaaaacg 1800	
cttaatgcct ggggatccct ctgcaactct cactgggaca tagaagatgc ccacgttctt 1860	
tgccaacaac ttaaagtgg agttgccctt tctaccccag gaggagcaca ttttgaaaaa 1920	
ggaaatggtc aggtctggag gcatatgttt cactgcaactg ggactgagca gcacatggga 1980	
gattgtcctg taactgctct ggggtgcttca ctatgtcctt cagggaagt ggcctctgta 2040	
atctgtcag gaaaccagt ccaaacactg tcctcgtgca attcatcatc tctgggcca 2100	
acaaggccta ccattccaga agaaagtgt gtggcctgca tagagagtgg tcaacttcgc 2160	
ttggtaaagt gaggaggtcg ctgtgctggg agagtagaga tttatcatga gggctcctgg 2220	
ggcaccatct gtgatgacag ctgggacctg agcgatgcc acgtggtgtg cagacagctg 2280	
ggctgtggag aggccattaa tgccactggg tctgtcatt ttggagaagg aacaggggcc 2340	
atctggctgg atgagatgaa atgcaatgga aaagaatccc gcatttggca gtgccattca 2400	
catggctggg ggcagcaaaa ctgcaggcac aaggaggatg caggagtat ctgtcagag 2460	
ttcatgtctc tgagactgac cagtgaagcc agcagagagg cctgtgcagg gcgtctagaa 2520	
gttttttaca acggagcttg gggcagtgtt ggcaggagta acatgtctga aaccactgtg 2580	
gggtgtgggt gcaggcagct gggctgtgca gacaaaggga aaatcaaccc tgcattctta 2640	
gacaaggcca tgtccattcc catgtgggtg gacaatgttc agtgtccaaa aggacctgac 2700	
acgtgtggc agtgcccatc atctccatgg gagaagagac tggccaggcc ctggaggag 2760	
acctggatca catgtgaca caagatgaga ctacaagaag gaccacttc ctgttctgga 2820	
cgtgtggaga tctggcacgg aggttcctgg gggacagtgt gtgatgactc ctgggacttg 2880	
aacgatgctc aggtggtgtg tcaacaactt ggctgtggtc cagctttgaa agcattcaaa 2940	
gaagcagagt ttggtcaggg gactggacct atatggctca atgaagtga gtgcaaaggg 3000	
aatgagtctt cctgtggga ttgtcctgcc agacgctggg gccacagtga gtgtggacac 3060	
aaggaagacg ctgcagtga ttgcacagat atttcaacga aaaaaacccc aaaaaagcc 3120	
acaacaggtc agtcatccct tattgcagtc ggaatccttg gagttgttct cttggtcatt 3180	
ttcgtcgcat tattcttgac tcaaaagcga agacagagac agcggttac agtttctca 3240	
agaggagaga acttagtcca ccaaattcaa taccgggaga tgaattcttg cctgaatgca 3300	
gatgatctgg acctaatgaa ttcctcagga ggccattctg aggcacactg aaaaggaaaa 3360	
tgggaattta taaccagtg agccttgaag ataccttgat gaagacctgg acta 3414	
1 M S K L R M V L L E D S G S A D V R R H 1 atgagcaaactcagaatgggtgctacttgaagactctggatctgctgacgtcagaagacat 21 F V N L S P F T I A V V L L L R A C F V 61 tttgtcaacttgagtccttctactattgctgtggtcttacttctcgtgcctgtttgtc 41 T S S L G G T T K E L R L V D G E N K C 121 accagttctcttgagggaacaaccaaggagctgaggctagtggatgggtgaaaacaagtgt 61 S G R V E V K I Q E E W G T V C N N G W	SEQ ID NO: 31 and 32

181 agtgggagagtgggaagtgaaaatccaggaggagtggggaacgggtgtgtaataatggctgg
 81 S M E A V S V I C N Q L G C P T A I K A
 241 agcatggaagcagtcctctgtgatttgaaccagctgggatgtccaactgctatcaaagcc
 101 T G W A N S S A G S G R I W M D H V S C
 301 actggatgggctaattccagttcaggttctggagcagcttggatggatcatgtttcttgt
 121 R G N E S A L W D C K H D G W G K H S N
 361 cgtgggaatgagtcagctccttgggactgcaaacatgatggatggggaagcatagtaac
 141 C T H Q Q D A G V T C S D G S D L E M R
 421 tgtactaccaacaagatgctggagtgacttgtcagatggatccgatttggaaatgagg
 161 L T N G G N M C S G R I E I K F Q G Q W
 481 ctgacgaatggaggggaatatgtgttctggaagaatagagatcaaattccaaggacagtgg
 181 G T V C D D N F N I N H A S V V C K Q L
 541 ggaacagtgtgtgatgataacttcaacatcaatcatgcatctgtggtttgtaacaactt
 201 E C G S A V S F S G S A N F G E G S G P
 601 gaatgtggaagtgtgtcagtttctctggttcagctaattttggagaaggctctggacca
 221 I W F D D L I C N G N E S A L W N C K H
 661 atctgggtttgatgatcttatatgcaacggaaatgagtcagctctctggaactgcaaacat
 241 Q G W G K H N C D H A E D A G V I C S K
 721 caaggatggggaagcataactgtgatcatgctgaggatgctggagtgtttgctcaaag
 261 G A D L S L R L V D G V T E C S G R L E
 781 ggagcagatctgagcctgagactggtagatggagtcactgaatgttcaggaagattagaa
 281 V R F Q G E W G T I C D D G W D S H D A
 841 gtgagattccaaggagaatgggggacaatatgtgatgacggctgggacagtcagatgct
 301 A V A C K Q L G C P T A I T A I G R V N
 901 gctgtggcatgcaagcaactgggactgccaactgctatcaccgccattgggtcgagttaac
 321 A S E G F G H I W L D S V S C Q G H E P
 961 gccagtgagggatttggacacatctggccttgacagtgtttcttgccaggacatgaacct
 341 A V W Q C K H H E W G K H Y C N H N E D
 1021 gcggtctggcaatgtaaacaccatgaatggggaagcattattgcaatcacaatgaagat
 361 A G V T C S D G S D L E L R L R G G G S
 1081 gctggcgtaacatgttctgatggatcagatctggagctaagacttagaggtggaggcagc
 381 R C A G T V E V E I Q R L L G K V C D R
 1141 cgctgtgctgggacagttgaggtggagattcagagactgttagggaaggtgtgtgacaga
 401 G W G L K E A D V V C R Q L G C G S A L
 1201 ggctggggactgaaagaagctgatgtggtttgcaggcagctgggatgtggatctgcactc
 421 K T S Y Q V Y S K I Q A T N M W L F L S
 1261 aaaacatcctatcaagtatactccaaaatccagggaacaaacatgtggctgtttctaagt
 441 S C N G N E T S L W D C K N W Q W G G L
 1321 agctgtaacggaaatgaaacttctcttgggactgcaagaactggcaatggggtggactt
 461 T C D H Y E E A K I T C S A H R E P R L
 1381 acctgtgatcactatgaagaagccaaattacctgtcagcccacagggaaaccagactg
 481 V G G D I P C S G R V E V K H G D T W G
 1441 gttggaggagacattccctgttctggacgcgttgaagtgaagcatggtgacacatggggc
 501 S V C D S D F S L E A A S V L C R E L Q
 1501 tccgtctgtgattcggatttctctctggaagctgccagcgttctatgcagggaattacag
 521 C G T V V S I L G G A H F G E G N G Q I
 1561 tgtggcacagtcgtctctatcctggggggagctcactttggagagggaaatggacagatc
 541 W A E E F Q C E G H E S H L S L C P V A
 1621 tgggctgaagaattccagtgtaggggacatgagtcctcttctcactctgcccagtagca
 561 P R P E G T C S H S R D V G V V C S R Y
 1681 ccccgccagaaggaactttagccacagcagggatgttggagtagtctgctcaagatac
 581 T E I R L V N G K T P C E G R V E L K T
 1741 acagaaattcgcttggatgaatggcaagaccccatgtgagggcagagtgagctcaaaacg
 601 L N A W G S L C N S H W D I E D A H V L
 1801 cttaatgcctggggatccctctgcaactctcactgggacatagaagatgccacggttctt
 621 C Q Q L K C G V A L S T P G G A H F G K
 1861 tgccaacaacttaaatgtggagtggcctttctacccaggaggagcacattttggaaaa
 641 G N G Q V W R H M F H C T G T E Q H M G
 1921 ggaaatggctcaggtctggaggcatatgtttcactgactgggactgagcagcacatggga
 661 D C P V T A L G A S L C P S G Q V A S V
 1981 gattgtcctgtaactgctctgggtgcttctcactatgtccttcagggcaagtggcctctgta
 681 I C S G N Q S Q T L S S C N S S S L G P
 2041 atttgctcaggaaccagtcctcaaacactgtcctcgtgcaattcatcatctctgggcca
 701 T R P T I P E E S A V A C I E S G Q L R
 2101 acaaggcctaccattccagaagaaagtgtgtggcctgcatagagagtggtcaacttcgc
 721 L V N G G G R C A G R V E I Y H E G S W
 2161 ttggtaaatggaggaggtcgctgtgctgggagagtagagatttatcatgagggctcctgg
 741 G T I C D D S W D L S D A H V V C R Q L
 2221 ggcaccatctgtgatgacagctgggacctgagcagatgccacgtggtgtgcagacagctg
 761 G C G E A I N A T G S A H F G E G T G P
 2281 ggctgtggagaggccattaatgccactggttctgctcattttggagaaggaacagggccc
 781 I W L D E M K C N G K E S R I W Q C H S
 2341 atctggctggatgagatgaaatgcaatggaaaagaatcccgatttggcagtgccattca
 801 H G W G Q Q N C R H K E D A G V I C S E
 2401 catggctggggcagcaaaactgcaggcacaaggaggatgcaggagttatctgctcagag
 821 F M S L R L T S E A S R E A C A G R L E
 2461 ttcattgtctctgagactgaccagtgaaagccagagagggcctgtgcagggcgtctagaa
 841 V F Y N G A W G S V G R S N M S E T T V
 2521 gttttttacaacggagcttggggcagtggtggcaggagtaacatgtctgaaaccactgtg
 861 G V V C R Q L G C A D K G K I N P A S L
 2581 ggtgtggtgtgcaggcagctgggctgtgcagacaaagggaatcaaccctgcatcttta
 881 D K A M S I P M W V D N V Q C P K G P D
 2641 gacaaggccatgtccattcccatgtgggtggacaatgttcagtgtccaaaaggacctgac

901 T L W Q C P S S P W E K R L A R P S E E 2701 acgctgtggcagtgcccatcatctccatgggagaagagactggccaggccctcggaggag 921 T W I T C D N K M R L Q E G P T S C S G 2761 acctggatcacatgtgacaacaagatgagactacaagaaggaccacttctgttctgga 941 R V E I W H G G S W G T V C D D S W D L 2821 cgtgtggagatctggcacggaggttcctgggggacagtgtgtgatgactcctgggacttg 961 N D A Q V V C Q Q L G C G P A L K A F K 2881 aacgatgctcaggtgggtgtgtcaacaacttggctgtgtgtccagctttgaaagcattcaaa 981 E A E F G Q G T G P I W L N E V K C K G 2941 gaagcagagtttgggtcaggggactggaccatattggctcaatgaagtgaagtgaaggg 1001 N E S S L W D C P A R R W G H S E C G H 3001 aatgagtcttcttctgtgggattgtcctgccagacgctggggccacagtgagtgtggacac 1021 K E D A A V N C T D I S T N K T P Q K A 3061 aaggaagacgctgcagtgaattgcacagatatttcaacgaacaaaacccacaaaaagcc 1041 T T G Q S S L I A V G I L G V V L L V I 3121 acaacaggtcagtcattcccttattgcagtcggaatccttggagttgttctcttgggtcatt 1061 F V A L F L T Q K R R Q R Q R L T V S S 3181 ttctgtcgattattcttctgactcaaaagcgaagacagagacagcggttacagtttctca 1081 R G E N L V H Q I Q Y R E M N S C L N A 3241 agaggagagaacttagtccaccaaattcaataccgggagatgaattcttgcctgaatgca 1101 D D L D L M N S S G G H S E A H 3301 gatgatctggacctaattgaattcctcaggaggccattctgaggcacac							
51	1	MSKLRMVLE	DSGSADVRRH	FVNLSPTTIA	VVLLLRACFV	TSSLGGTTKE	SEQ ID NO: 32
101		LRLVDGENKC	SGRVEVKIQE	EWGTVCNNGW	SMEAVSVICN	QLGCPTAIKA	
151		TGWANSSAGS	GRIWMDHVSC	RGNESALWDC	KHDGWGKHSN	CTHQQDAGVT	
201		CSDGSDLEMR	LTNGGNMCSG	RIEIKFQGW	GTVCDDNFNI	NHASVVCKQL	
251		ECGSAVSFSG	SANFGEESGP	IWFDDLICNG	NESALWNCKH	QGWGKHNCNH	
301		AEDAGVICK	GADLSLRLVD	GVTECSGRLE	VRFQGEWGTI	CDDGWDSDHA	
351		AVACKQLGCP	TAITAIGRVN	ASEGFGHIWL	DSVSCQGHEP	AVWQCKHHEW	
401		GKHYCNHNE	AGVTCSDGSD	LELRLRGGS	RCAGTVEVEI	QRLLGKVCDR	
451		GWGLKEADV	CRQLGCGSAL	KTSYQVYSKI	QATNMWLFLS	SCNGNETSLW	
501		DCKNWQWGL	TCDHYEEAKI	TCSAHREPL	VGGDIPCSGR	VEVKHGDTHG	
551		SVCDSDFSLE	AASVLCRELQ	CGTVVSILGG	AHFGEENGQI	WAEFQCEGH	
601		ESHLSLCPVA	PRPEGTCSSH	RDVGVCSSRY	TEIRLVNGKT	PCEGRVELKT	
651		LNAWGSLCNS	HWDIEDAHL	CQQLKCGVAL	STPGGAHFGK	GNGQVWRHMF	
701		HCTGTQHMG	DCPVTALGAS	LCPGQVASV	ICSGNQSQT	LSSCNSSSLGP	
751		TRPTIPEESA	VACIESGQLR	LVNGGRCAG	RVEIYHEGSW	GTICDDSWDL	
801		SDAHVVCRL	GCGEAINATG	SAHFGEGTGP	IWLDEMKNCG	KESRIWQCHS	
851		HGWGQONCRH	KEDAGVICSE	FMSLRLTSEA	SREACAGRLE	VFYNGAWGSV	
901		GRSNMSETTV	GVVCRLGCA	DKGKINPASL	DKAMSIPMWV	DNVQCPKGP	
951		TLWQCPSSPW	EKRLARPSEE	TWITCDNMR	LQEGPTSCSG	RVEIWHGGSW	
1001		GTVCDDSWDL	NDAQVVCQL	GCGPALKAFK	EAEFGQGTGP	IWLNEVKCKG	
1051		NESSLWDCA	RRWGHSECGH	KEDAAVNCTD	ISTNKTPQKA	TTGQSSLIIV	
1101		GILGVLLVI	FVALFLTQKR	RQRQLTVSS	RGENLVHQIQ	YREMNSCLNA	
		DDLMLNSSG	GHSEAH				

Example 9. Cloning and characterization of simian CD163 from Vero cells.

A forward primer 5'simianCD163 (SEQ ID NO: 28) (5'-CACCGGAATGAGCAAACCTCAGAATGG-3' based on human CD163) and a reverse primer HuCD163-3'Kpn (SEQ ID NO:29) (5'-

- 5 TGCTCCGGTACCTAGTCCAGGTCTTCATCAAGGTATCTTA-3') were used to amplify CD163 cDNA from Vero cells. Total cellular RNA was prepared from Vero cells using the RNeasy kit. RT-PCR parameters were the same as described in Example 4. RT-PCR products were cloned directionally into the pCDNA3.1D/V5/His/TOPO vector according to the manufacturer's instruction. Eight clones containing large inserts were sequenced, and six discrete splicing patterns were found. These patterns are depicted graphically in Figure 17.

The six splicing variants differ in the presence or absence of three exons, designated E6, E105, and E83. Omissions of E6 or E105 do not change the reading

frame, whereas omission of E83 does. Patterns similar to v2 and/or v3 were also seen in porcine, murine, human, and MARC-145 monkey cells. Patterns v4 and v5 lack the 105-nucleotide exon that encodes the hydrophobic transmembrane region. These cDNAs were unable to render BHK cells permissive to PRRSV infection in a transient
 5 transfection assay, probably because the CD163 is secreted rather than remaining membrane bound. Although CD163 molecules lacking a transmembrane region appear to be non-functional as cellular permissivity factors, it is possible that they may have utility either in direct virus neutralization (similar to neutralizing antibodies), or as an immunogen for the induction of anti-CD163 antibodies which would block viral
 10 infection in the host animal.

The longest splice variant, v7, contains all three of the exons E6, E105, and E83. This novel CD163 cDNA from Vero cells encodes a polypeptide that is 1153 amino acids in length. When compared to the sequences in Genbank database, the Vero CD163v7 amino acid sequence is 95.4% identical to human CD163 (Genbank Z22968),
 15 83.7% identical to pig CD163 (Genbank AJ311716), and 72.1% identical to mouse CD163 (Genbank AF274883). The nucleotide and amino acid sequences of the six splice variants found in Vero cells are provided below (SEQ ID NOS:33-44).

SEQUENCE	ID NO
1 M S K L R M V L L E D S G S A D V R R H 1 ATGAGCAAACCTCAGAATGGTGCTACTTGAAGACTCTGGATCTGCTGACGTCAGAAGACAT 21 F V N L S P F T I A V V L L L R A C F V 61 TTTGTCAACTTGAGTCCCTTCACTATTGCTGTGGTCTTACTTCTCCGTGCCTGTTTTGTGTC 41 T S S L G G T T K E L R L V D G E N K C 121 ACCAGTTCTCTTGGAGGAACAACCAAGGAGCTGAGGCTAGTGGATGGTGAAAACAAGTGT 61 S G R V E V K I Q E E W G T V C N N G W 181 AGTGGGAGAGTGGAAGTGAAATCCAGGAGGAGTGGGGAACGGTGTGTAATAATGGCTGG 81 S M E A V S V I C N Q L G C P T A I K A 241 AGCATGGAAGCAGTCTCTGTGATTTGTAACCAGCTGGGATGTCCAAGTCTATCAAAGCC 101 T G W A N S S A G S G R I W M D H V S C 301 ACTGGATGGGCTAATTCCAGTGCAGGTTCTGGACGCATTTGGATGGATCATGTTTCTTGT 121 R G N E S A L W D C K H D G W G K H S N 361 CGTGGGAATGAGTCAGCTCTTTGGGACTGCAAACATGATGGATGGGGAAAGCATAGTAAC 141 C T H Q Q D A G V T C S D G S D L E M R 421 TGTACTCACCAACAAGATGCTGGAGTAACTTGCTCAGATGGATCCGATTTGGAAATGAGG 161 L T N G G N M C S G R I E I K F Q G Q W 481 CTGACGAATGGAGGGAATATGTGTTCTGGAAGAATAGAGATCAAATTCGAAGGACAGTGG 181 G T V C D D N F N I N H A S V V C K Q L 541 GGAACAGTGTGTGATGATAACTTCAACATCAATCATGCATCTGTGGTTTGTAAACAACTT 201 E C G S A V S F S G S A N F G E G S G P 601 GAATGTGGAAGTGCTGTGCTAGTTTCTCTGGTTCAGCTAATTTTGGAGAAGGCTCTGGACCA 221 I W F D D L I C N G N E S A L W N C K H 661 ATCTGGTTTGATGATCTTATATGCAACGGAAATGAGTCAGCTCTCTGGAAGTGCAAACAT	SEQ ID NO:33 and 34

241 Q G W G K H N C D H A E D A G V I C S K
 721 CAAGGATGGGGAAAGCATAACTGTGATCATGCTGAGGATGCTGGAGTGATTTGCTCAAAG
 261 G A D L S L R L V D G V T E C S G R L E
 781 GGAGCAGATCTGAGCCTGAGACTGGTAGATGGAGTCACTGAATGTTTCAGGAAGATTAGAA
 281 V R F Q G E W G T I C D D G W D S H D A
 841 GTGAGATTCCAAGGAGAATGGGGGACAATATGTGATGACGGCTGGGACAGTCATGATGCT
 301 A V A C K Q L G C P T A I T A I G R V N
 901 GCTGTGGCATGCAAGCAACTGGGATGTCCAAGTCTATCACCGCCATTGGTTCGAGTTAAC
 321 A S E G F G H I W L D S V S C Q G H E P
 961 GCCAGTGAGGGATTTGGACACATCTGGCTTGACAGTGTTTCTTGCCAGGGACATGAACCT
 341 A V W Q C K H H E W G K H Y C N H N E D
 1021 GCGGTCTGGCAATGTAAACACCATGAATGGGGAAAGCATTATTGCAATCACAATGAAGAT
 361 A G V T C S D G S G L E L R L R G G G S
 1081 GCTGGCGTAACATGTTCTGATGGATCAGGTCTGGAGCTAAGACTTAGAGGTGGAGGCAGC
 381 R C A G T V E V E I Q R L L G K V C D R
 1141 CGCTGTGCTGGGACAGTTGAGGTGGAGATTGAGAGACTGTTAGGGAAGGTGTGTGACAGA
 401 G W G L K E A D V V C R Q L G C G S A L
 1201 GGCTGGGGACTGAAAGAAGCTGATGTGGTTTGCAGGCAGCTGGGATGTGGATCTGCACTC
 421 K T S Y Q V Y S K I Q A T N M W L F L S
 1261 AAAACATCCTATCAAGTATACTCCAAAATCCAGGCAACAAACATGTGGCTGTTTCTAAGT
 441 S C N G N E T S L W D C K N W Q W G G L
 1321 AGCTGTAACGGAAATGAACTTCTCTTTGGGACTGCAAGAACTGGCAATGGGGTGGACTT
 461 T C D H Y E E A K I T C S A H R E P R L
 1381 ACCTGTGATCACTATGAAGAAGCCAAAATTACCTGCTCAGCCACAGGGAACCCAGACTG
 481 V G G D I P C S G R V E V K H G D T W G
 1441 GTTGGAGGAGACATTCCCTGTTCTGGACGCGTTGAAGTGAAGCATGGTGACACATGGGGC
 501 S V C D S D F S L E A A S V L C R E L Q
 1501 TCCGTCTGTGATTCGGATTTCTCTCTGGAAGCTGCCAGCGTTCTATGCAGGGAATTACAG
 521 C G T V V S I L G G A H F G E G N G Q I
 1561 TGTGGCACAGTCGTCTCTATCCTGGGGGGAGCTCACTTTGGAGAGGGAAATGGACAGATC
 541 W T E E F Q C E G H E S H L S L C P V A
 1621 TGGACTGAAGAATTCCAGTGTGAGGGACATGAGTCCCATCTTTCACCTCTGCCCAGTAGCA
 561 P R P E G T C S H S R D V G V V C S R Y
 1681 CCCCAGCCAGAAAGGAACCTGTAGCCACAGCAGGGATGTTGGAGTAGTCTGCTCAAGATAC
 581 T E I R L V N G K T P C E G R V E L K T
 1741 ACAGAAATTCGCTTGGTGAATGGCAAGACCCCATGTGAGGGCAGAGTGGAGCTCAAAACG
 601 L N A W G S L C N S H W D I E D A H V L
 1801 CTTAATGCCTGGGGATCCCTCTGCAACTCTCACTGGGACATAGAAGATGCCACGTTCTT
 621 C Q Q L K C G V A L S T P G G A H F G K
 1861 TGCCAACAACCTAAATGTGGAGTTGCCCTTCTACCCAGGAGGAGCACATTTTGGAAAA
 641 G N G Q V W R H M F H C T G T E Q H M G
 1921 GGAAATGGTCAGGTCTGGAGGCATATGTTTCACTGCACTGGGACTGAGCAGCACATGGGA
 661 D C P V T A L G A S L C P S G Q V A S V
 1981 GATTGTCCTGTAAGTCTGCTGGGTGCTTCACTATGTCCTTCAGGGCAAGTGGCCTCTGTA
 681 I C S G N Q S Q T L S S R N S S S L G P
 2041 ATTTGCTCAGGAAACCAGTCCCAAACACTGTCCTCGCGCAATTCATCATCTCTGGGCCCA
 701 T R P T I P E E S A V A C I E S G Q L R
 2101 ACAAGGCCTACCATTCCAGAAGAAAGTGCTGTGGCCTGCATAGAGAGTGGTCAACTTCGC
 721 L V N G G G R C A G R V E I Y H E G S W
 2161 TTGGTAAATGGAGGAGGTCGCTGTGCTGGGAGAGTAGAGATTTATCATGAGGGCTCCTGG
 741 G T I C D D S W D L S D A H V V C R Q L
 2221 GGCACCATCTGTGATGACAGCTGGGACCTGAGCGATGCCACGTGGTGTGCAGACAGCTG
 761 G C G E A I N A T G S A H F G E G T G P
 2281 GGCTGTGGAGAGGCCATTAATGCCACTGGTTCTGCTCATTTTGGAGAAGGAACAGGGCCC
 781 I W L D E M K C N G K E S R I W Q C H S

2341 ATCTGGCTGGATGAGATGAAATGCAATGGAAAAAGATCCCGCATTGTCAGTGCCATTCA 801 H G W G Q Q N C R H K E D A G V I C S E 2401 CATGGCTGGGGGCAGCAAAACTGCAGGCACAAGGAGGATGCAGGAGTTATCTGCTCAGAG 821 F M S L R L T S E A S R E A C A G R L E 2461 TTCATGTCTCTGAGACTGACCAGTGAAGCCAGCAGAGAGGCCTGTGCAGGGCGTCTAGAA 841 V F Y N G A W G S V G R S N M S E T T V 2521 GTTTTTTACAACGGAGCTTGGGGCAGTGTGGCAGGAGTAACATGTCTGAAACCACTGTG 861 G V V C R Q L G C A D K G K I N S A S L 2581 GGTGTAGTGTGCAGGCAGCTGGGCTGTGCAGACAAAGGGAAAATCAACTCTGCATCTTTA 881 D K A M S I P M W V D N V Q C P K G P D 2641 GACAAGGCCATGTCCATTCCCATGTGGGTGGACAATGTTTCAGTGTCCAAAAGGACCTGAC 901 T L W Q C P S S P W E K R L A R P S E E 2701 ACGCTGTGGCAGTGCCCATCATCTCCATGGGAGAAGAGACTGGCCAGGCCCTCGGAGGAG 921 T W I T C D N K M R L Q E G P T S C S G 2761 ACCTGGATCACATGTGACAACAAGATGAGACTACAAGAAGGACCCACTTCCTGTTCTGGA 941 R V E I W H G G S W G T V C D D S W D L 2821 CGTGTGGAGATCTGGCACGGAGGTTCTGGGGGACAGTGTGTGATGACTCCTGGGACTTG 961 N D A Q V V C Q Q L G C G P A L K A F K 2881 AACGATGCTCAGGTGGTGTGTCAACAACCTGGCTGTGGTCCAGCTTTGAAAGCATTCAAA 981 E A E F G Q G T G P I W L N E V K C E G 2941 GAAGCAGAGTTTGGTCAGGGGACTGGACCCATATGGCTCAATGAAGTGAAGTGCGAAGGG 1001 N E S S L W D C P A R R W G H S E C G H 3001 AATGAGTCTTCCTTGTGGGATTGTCTGCCAGACGCTGGGGCCACAGTGAGTGTGGACAC 1021 K E D A A V N C T D I S T R K T P Q K A 3061 AAGGAAGACGCTGCAGTGAATTGCACAGATATTTCAACGCGCAAAACCCACAAAAAGCC 1041 T T G Q S S L I A V G I L G V V L L A I 3121 ACAACAGGTCAATCATCCCTTATTGCAGTCGGAATCCTTGGAGTTGTTCTCTTGGCCATT 1061 F V A L F L T Q K R R Q R Q R L T V S S 3181 TTCGTGCGATTATTCTTGAATCAAAAGCGAAGACAGAGACAGCGGCTTACAGTTTCCTCA 1081 R G E N L V H Q I Q Y R E M N S C L N A 3241 AGAGGAGAGAACTTAGTCCACCAAATTCATACCGGGAGATGAATTCTTGCTGAATGCA 1101 D D L D L M N S S G G H S E A H 3301 GATGATCTGGACCTAATGAATTCCTCAGGAGGCCATTCTGAGGCACAC	
1 MSKLRLVLE DSGSADVRH FVNLSPFTIA VLLLRACFV TSSLGTTKE LRLVDGENKC 61 SGRVEVKIQE EWGTVCNNGW SMEAVSVICN QLGCPAIAK TGWANSSAGS GRIWMDHVSC 121 RGNESALWDC KHDGWGKHSN CTHQQDAGVT CSDGSDLEMR LTNGGNMCSG RIEIKFQGW 181 GTVCDDNFNI NHASVVCQQL ECGSAVSFSG SANFGECSGP IWFDDLICNG NESALWNCKH 241 QGWGKHNCNDH AEDAGVICSK GADLSLRLVD GVTECSGRLE VRFQGEWGTI CDDGWDSDHA 301 AVACKQLGCP TAITAIGRVN ASEGFHGIWL DSVSCQGHEP AVWQCKHHEW GKHYCNHNE 361 AGVTCSDGSG LELRLRGGGS RCAGTVEVEI QRLGKVCDR GWGLKEADV CRQLGCGSAL 421 KTSYQVYSKI QATNMWLFSL SCNGNETSLW DCKNWQWGL TCDHYEEAKI TCSAHREPR 481 VGGDIPCSGR VEVKHGDTWG SVCDSDFSLE AASVLCRELQ CGTVVSILGG AHFGEENGQI 541 WTEEFQCEGH ESHLSLCPVA PRPEGTCSSH RDVGVCVSRV TEIRLVNGKT PCEGRVELKT 601 LNAWGLCNS HWDIEDAHLV CQQLKCGVAL STPGGAHFGK GNGQVWRHMF HCTGTEQHMG 661 DCPVTALGAS LCPSGQVASV ICSGNQSQTL SSRNSSLGP TRPTIPEESA VACIESGQLR 721 LVNGGGRGAG RVEIYHEGSW GTICDDSWDL SDAHVVCRQL GCGEAINATG SAHFGEETGP 781 IWLDEMKNCG KESRIWQCHS HGWGQONCRH KEDAGVICSE FMSLRILTSEA SREACAGRLE 841 VFYNGAWGSV GRSNMSETTV GVVCRLGCA DKGKINSASL DKAMSIPMWV DNVQCPKGP 901 TLWQCPSSPW EKRLARPSEE TWITCDNKM LQEGPTSCSG RVEIWHGGSW GTVCDDSWDL 961 NDAQVVCQQL GCGPALKAFK EAEFGQGTGP IWLNEVKCEG NESSLWDCA RRWGHSECGH 1021 KEDAAVNCTD ISTRKTPQKA TTGQSSLIIV GILGVLLAI FVALFLTQKR RQRRLTVSS 1081 RGENLVHQIQ YREMNSCLNA DDLDLMNSSG GHSEAH	SEQ ID NO:34
1 M S K L R M V L L E D S G S A D V R R H 1 ATGAGCAAACTCAGAATGGTGCTACTTGAAGACTCTGGATCTGCTGACGTCAGAAGACAT 21 F V N L S P F T I A V V L L L R A C F V 61 TTTGTCAACTTGAGTCCCTTCACTATTGCTGTGGTCTTACTTCTCCGTGCCTGTTTTGTC 41 T S S L G G T T K E L R L V D G E N K C 121 ACCAGTCTCTTGGAGGAACAACCAAGGAGCTGAGGCTAGTGGATGGTGAAAACAAGTGT 61 S G R V E V K I Q E E W G T V C N N G W 181 AGTGGGAGAGTGGAAGTGAAAATCCAGGAGGAGTGGGGAACGGTGTGTAATAATGGCTGG	SEQ ID NO:35 and 36

81 S M E A V S V I C N Q L G C P T A I K A
 241 AGCATGGAAGCAGTCTCTGTGATTTGTAACCAGCTGGGATGTCCAAGTCTATCAAAGCC
 101 T G W A N S S A G S G R I W M D H V S C
 301 ACTGGATGGGCTAATTCCAGTGCAGGTTCTGGACGCATTTGGATGGATCATGTTTCTTGT
 121 R G N E S A L W D C K H D G W G K H S N
 361 CGTGGGAATGAGTCAGCTCTTTGGGACTGCAAACATGATGGATGGGAAAGCATAGTAAC
 141 C T H Q Q D A G V T C S D G S D L E M R
 421 TGTACTCACCACAAGATGCTGGAGTAAGTCTGCTCAGATGGATCCGATTTGGAAATGAGG
 161 L T N G G N M C S G R I E I K F Q G Q W
 481 CTGACGAATGGAGGGAATATGTGTTCTGGAAGAATAGAGATCAAATTTCCAAGGACAGTGG
 181 G T V C D D N F N I N H A S V V C K Q L
 541 GGAACAGTGTGTGATGATAACTTCAACATCAATCATGCATCTGTGGTTTGTAAACAACCTT
 201 E C G S A V S F S G S A N F G E G S G P
 601 GAATGTGGAAGTGTCTGCTCAGTTTCTCTGTTCTAGCTAATTTTGGAGAAGGCTCTGGACCA
 221 I W F D D L I C N G N E S A L W N C K H
 661 ATCTGGTTTGTGATGATCTTATATGCAACGGAATGAGTCAGCTCTCTGGAAGTCAAACAT
 241 Q G W G K H N C D H A E D A G V I C S K
 721 CAAGGATGGGGAAGCATAACTGTGATCATGCTGAGGATGCTGGAGTGATTTGCTCAAAG
 261 G A D L S L R L V D G V T E C S G R L E
 781 GGAGCAGATCTGAGCCTGAGACTGGTAGATGGAGTCACTGAATGTTTCAAGGAAGATTAGAA
 281 V R F Q G E W G T I C D D G W D S H D A
 841 GTGAGATTCCAAGGAGAATGGGGGACAATATGTGATGACGGCTGGGACAGTCATGATGCT
 301 A V A C K Q L G C P T A I T A I G R V N
 901 GCTGTGGCATGCAAGCAACTGGGATGTCCAAGTCTATCACCAGCATTGGTCGAGTTAAC
 321 A S E G F G H I W L D S V S C Q G H E P
 961 GCCAGTGAGGGATTTGGACACATCTGGCTTGACAGTGTCTTCTGCCAGGGACATGAACCT
 341 A V W Q C K H H E W G K H Y C N H N E D
 1021 GCGGTCTGGCAATGTAAACACCATGAATGGGGAAGCATTATTGCAATCACAATGAAGAT
 361 A G V T C S D G S D L E L R L R G G G S
 1081 GCTGGCGTAACATGTTCTGATGGATCAGATCTGGAGCTAAGACTTAGAGGTGGAGGCAGC
 381 R C A G T V E V E I Q R L L G K V C D R
 1141 CGCTGTGCTGGGACAGTTGAGGTGAGATTGAGAGCTGTTAGGGAAGGTGTGTGACAGA
 401 G W G L K E A D V V C R Q L G C G S A L
 1201 GGCTGGGGACTGAAAGAAGCTGATGTGTTTGCAGGCAGCTGGGATGTGGATCTGCACTC
 421 K T S Y Q V Y S K I Q A T N M W L F L S
 1261 AAAACATCCTATCAAGTATACTCCAAATCCAGGCAACAAACATGTGGCTGTTTCTAAGT
 441 S C N G N E T S L W D C K N W Q W G G L
 1321 AGCTGTAACGGAATGAACTTCTCTTGGGACTGCAAGAAGTGGCAATGGGGTGGACTT
 461 T C D H Y E E A K I T C S A H R E P R L
 1381 ACCTGTGATCACTATGAAGAAGCCAAATACCTGCTCAGCCCACAGGGAACCCAGACTG
 481 V G G D I P C S G A R V E V K H G D T W G
 1441 GTTGGAGGAGACATTCCCTGTTCTGGACGCGTTGAAGTGAAGCATGGTGACACATGGGGC
 501 S V C D S D F S L E A A S V L C R E L Q
 1501 TCCGTCTGTGATTCGGATTTCTCTCTGGAAGCTGCCAGCGTTCTATGCAGGGAATTACAG
 521 C G T V V S I L G G A H F G E G N G Q I
 1561 TGTGGCACAGTCGTCTCTATCCTGGGGGGAGCTCACTTTGGAGAGGGAATGGACAGATC
 541 W T E E F Q C E G H E S H L S L C P V A
 1621 TGGACTGAAGAATTCAGTGTGAGGGACATGAGTCCCATCTTTCACTCTGCCCAGTAGCA
 561 P R P E G T C S H S R D V G V V C S R Y
 1681 CCCCAGCCAGAAGGAAGTGTAGCCACAGCAGGGATGTTGGAGTAGTCTGCTCAAGATAC
 581 T E I R L V N G K T P C E G R V E L K T
 1741 ACAGAAATTCGCTTGGTGAATGGCAAGACCCCATGTGAGGGCAGAGTGGAGCTCAAAACG
 601 L N A W G S L C N S H W D I E D A H V L
 1801 CTTAATGCCTGGGGATCCCTCTGCAACTCTCACTGGGACATAGAAGATGCCACGTTCTT
 621 C Q Q L K C G V A L S T P G G A H F G K
 1861 TGCCAACAACCTTAAATGTGGAGTTGCCCTTTCTACCCAGGAGGAGCACATTTTGGAAAA
 641 G N G Q V W R H M F H C T G T E Q H M G
 1921 GGAAATGGTCAGGTCTGGAGGCATATGTTTCACTGCACTGGGACTGAGCAGCACATGGGA
 661 D C P V T A L G A S L C P S G Q V A S V
 1981 GATTGTCCTGTAAGTCTCTGGGTGCTTCACTATGTCCTTCAGGGCAAGTGGCCTCTGTA
 681 I C S G N Q S Q T L S S C N S S S L G P
 2041 ATTTGCTCAGGAAACAGTCCCAAACTGTCTCTGCTGCAATTCATCATCTCTGGGCCCA
 701 T R P T I P E E S A V A C I E S G Q L R
 2101 ACAAGGCCTACCATTCAGAAGAAAGTGTGTGGCCTGCATAGAGAGTGGTCAACTTCGC
 721 L V N G G G R C A G R V E I Y H E G S W
 2161 TTGGTAAATGGAGGAGGTGCTGTGCTGGGAGAGTAGAGATTTATCATGAGGGCTCCTGG
 741 G T I C D D S W D L S D A H V V C R Q L
 2221 GGCACCATCTGTGATGACAGCTGGGACCTGAGCGATGCCACGTGGTGTGACAGAGCTG
 761 G C G E A I N A T G S A H F G E G T G P
 2281 GGCTGTGGAGAGGCCATTAATGCCACTGGTTCTGCTCATTTTGGAGAAGGAACAGGGCCC
 781 I W L D E M K C N G K E S R I W Q C H S
 2341 ATCTGGCTGGATGAGATGAAATGCAATGGAAAAGAAATCCCGCATTTGGCAGTGCCATTCA
 801 H G W G Q Q N C R H K E D A G V I C S E
 2401 CATGGCTGGGGCAGCAAACTGAGGACAGGAGGATGCAGGAGTTATCTGCTCAGAG
 821 F M S L R L T S E A S R E A C A G R L E
 2461 TTCATGTCTCTGAGACTGACAGTGAAGCCAGCAGAGAGGCTGTGAGGGCGTCTAGAA
 841 V F Y N G A W G S V G R S N M S E T T V
 2521 GTTTTTTACAACGGAGCTTGGGGCAGTGTGGCAGGAGTAACATGTCTGAAACCACTGTG
 861 G V V C R Q L G C A D K G K I N S A S L
 2581 GGTGTAGTGTGAGGAGCTGGGCTGTGAGACAAAGGGAAAATCAACTCTGCATCTTTA
 881 D K A M S I P M W V D N V Q C P K G P D
 2641 GACAAGGCCATGTCCATTCCCATGTGGGTGGACAATGTTTCAAGTGTCCAAAAGGACCTGAC
 901 T L W Q C P S S P W E K R L A R P S E E

2701 ACGCTGTGGCAGTGGCCATCATCTCCATGGGAGAAGAGACTGGCCAGGCCCTCGGAGGAG 921 T W I T C D N K M R L Q E G P T S C S G 2761 ACCTGGATCACATGTGACAACAAGATGAGACTACAAGAAGGACCCACTTCCTGTTCTGGA 941 R V E I W H G G S W G T V C D D S W D L 2821 CGTGTGGAGATCTGGCACGGAGGTTCTGGGGGACAGTGTGTGATGACTCCTGGGACTTG 961 N D A Q V V C Q Q L G C G P A L K A F K 2881 AACGATGCTCAGGTGGTGTGTCAACAACCTGGCTGTGGTCCAGCTTTGAAAGCATTCAAA 981 E A E F G Q G T G P I W L N E V K C K G 2941 GAAGCAGAGTTTGGTCAGGGGACTGGACCCATATGGCTCAATGAAGTGAAGTGCAAAGGG 1001 N E S S L W D C P A R R W G H S E C G H 3001 AATGAGTCTTCCTTGTGGGATTGTCCTGCCAGACGCTGGGGCCACAGTGAGTGTGGACAC 1021 K E D A A V N C T D I S T R K T P Q K A 3061 AAGGAAGACGCTGCAGTGAATTGCACAGATATTTCAACGCGCAAAACCCACAAAAAGCC 1041 T T G Q S S L I A V G I L G V V L L A I 3121 ACAACAGGTCAAGTCATCCCTTATTGCAGTCGGAATCCTTGGAGTTGTTCTCTTGGCCATT 1061 F V A L F L T Q K R R Q R Q R L T V S S 3181 TTCGTGCAATTATTCTTGAAGCAGAGACAGACAGCGGCTTACAGTTTCCTCA 1081 R G E N L V H Q I Q Y R E M N S C L N A 3241 AGAGGAGAGAACTTAGTCCACCAATTCAATACCGGGAGATGAATTCTTGCCTGAATGCA 1101 D D L D L M N S S E N S N E S A D F N A 3301 GATGATCTGGACCTAATGAATTCCTCAGAAAATTCCAATGAGTCAGCTGATTTCAATGCT 1121 A E L I S V S K F L P I S G M E K E A I 3361 GCTGAACATAATTTCTGTGTCTAAATTTCTTCTTCTGGAATGGAAGAGGCCATT 1141 L R H T E K E N G N L 3421 CTGAGGCACACTGAAAAGGAAAATGGGAATTTA	
1 MSKLRMVLLE DSGSADVRRH FVNLSPFTIA VLLLLRACFV TSSLGGTTKE LRLVDGENKC 61 SGRVEVKIQE EWGTVCNNGW SMEAVSVICN QLGCPAIIKA TGWANSSAGS GRIWMDHVSC 121 RGNESALWDC KHDGWGKHSN CTHQQDAGVT CSDGSDLEMR LTNGGNMCSG RIEIKFQQGW 181 GTVCDDNFNI NHASVVCQQL ECGSAVSFSG SANFEGSGP IWFDDLICNG NESALWNCKH 241 QGWGKHNCNDH AEDAGVICS KADLSLRLVD GVTECSGRLE VRFQGEWGTI CDDGWDSDHA 301 AVACKQLGCP TAITAIGRVN ASEGFGHIWL DSVSCQHEP AVWQCKHHEW GKHYCNHND 361 AGVTCSDGSD LELRLRGGGS RCAGTVEVEI QRLLGKVC DR GWGLKEADV CRQLGCGSAL 421 KTSYQVYSKI QATNMWLFSL SCNGNETSLW DCKNWQWGL TCDHYEEAKI TCSAHREPL 481 VGGDIPCSGR VEVKHGDTWG SVCDSDFSLE AASVLCRELQ CGTVVSILGG AHFGEENGQI 541 WTEEFQCEGH ESHLSLCPVA PRPEGTCSSHS RDVGVCVCSRY TEIRLVNGKT PCEGRVELKT 601 LNAWGLSCLNS HWDIEDAHLV CQQLKCGVAL STPGGAHFGK GNGQVWRHMF HCTGTEQHMG 661 DCPVTALGAS LCPSGQVASV ICSGNQSQTL SSCNSSSLGP TRPTIPEESA VACIESGQLR 721 LVNGGGRCAG RVEIYHEGSW GTICDDSWDL SDAHVVCRQL GCGEAINATG SAHFEGGTGP 781 IWLDEMKNCG KESRIWQCHS HGWGQONCRH KEDAGVICSE FMSLRILTSEA SREACAGRL 841 VFYNGAWGSV GRSNMSETTV GVVCRLGCA DKGKINSASL DKAMSIPMWV DNVQCPKGP 901 TLWQCPSSPW EKRLARPSEE TWITCDNKM LQEGPTSCSG RVEIWHGGSW GTVCDDSWDL 961 NDAQVVCQQL GCGPALKAFK EAEFGQTGP IWLNEVKCKG NESSLWDCA RRWGHSECGH 1021 KEDAAVNCTD ISTRKTPQKA TTGQSSLIIV GILGVLLAI FVALFLTQKR RQRQLTVSS 1081 RGENLVHQIQ YREMNSCLNA DDLDLMNSSE NSNESADFNA AELISVSKFL PISGMEKEAI 1141 LRHTEKENG L	SEQ ID NO:36
1 M S K L R M V L L E D S G S A D V R R H 1 ATGAGCAAACTCAGAATGGTGTCTACTTGAAGACTCTGGATCTGCTGACGTCAGAAGACAT 21 F V N L S P F T I A V V L L L R A C F V 61 TTTGTCAACTTGAGTCCCTTCACTATTGCTGTGGTCTTACTTCTCCGTGCCTGTTTGTGTC 41 T S S L G G T T K E L R L V D G E N K C 121 ACCAGTTCTCTTGGAGGAACAACCAAGGAGCTGAGGCTAGTGGATGGTGAAAACAAGTGT 61 S G R V E V K I Q E E W G T V C N N G W 181 AGTGGGAGAGTGGAAGTGAAATCCAGGAGGAGTGGGGAACGGTGTGTAATAATGGCTGG 81 S M E A V S V I C N Q L G C P T A I K A 241 AGCATGGAAGCAGTCTCTGTGATTTGTAACCAGCTGGGATGTCCAAGTCTATCAAAGCC 101 T G W A N S S A G S G R I W M D H V S C 301 ACTGGATGGGCTAATTCAGTGCAGGTTCTGGACGCATTTGGATGGATCATGTTTCTTGT 121 R G N E S A L W D C K H D G W G K H S N 361 CGTGGGAATGAGTCAGCTCTTGGGACTGCAACATGATGGATGGGGAAGCATAGTAAC 141 C T H Q Q D A G V T C S D G S D L E M R 421 TGTACTCACCAACAAGATGCTGGAGTAACTTGCTCAGATGGATCCGATTTGGAAATGAGG 161 L T N G G N M C S G R I E I K F Q G Q W 481 CTGACGAATGGAGGGAATATGTGTTCTGGAAGAATAGAGATCAAAATCCAAGGACAGTGG 181 G T V C D D N F N I N H A S V V C K Q L 541 GGAACAGTGTGTGATGATAACTTCAACATCAATCATGCATCTGTGGTTTGTAAACAATT 201 E C G S A V S F S G S A N F G E G S G P 601 GAATGTGGAAGTGCTGTGAGTTTCTGTTTCTGAGCTAATTTTGGAGAAGGCTCTGGACCA 221 I W F D D L I C N G N E S A L W N C K H 661 ATCTGGTTTGTGATGATCTTATATGCAACGGAAATGAGTCAGCTCTCTGGAAGTCAACAT	SEQ ID NO:37 and 38

241 Q G W G K H N C D H A E D A G V I C S K
 721 CAAGGATGGGAAAGCATAACTGTGATCATGCTGAGGATGCTGGAGTGATTTGCTCAAAG
 261 G A D L S L R L V D G V T E C S G R L E
 781 GGAGCAGATCTGAGCCTGAGACTGGTAGATGGAGTCACTGAATGTTTACAGGAAGATTAGAA
 281 V R F Q G E W G T I C D D G W D S H D A
 841 GTGAGATTCCAAGGAGAATGGGGGACAATATGTGATGACGGCTGGGACAGTCATGATGCT
 301 A V A C K Q L G C P T A I T A I G R V N
 901 GCTGTGGCATGCAAGCAACTGGGATGTCCAAGTCTATCACCGCCATTGGTTCGAGTTAAC
 321 A S E G F G H I W L D S V S C Q G H E P
 961 GCCAGTGAGGGATTTGGACACATCTGGCTTGACAGTGTTCCTTGCCAGGGACATGAACCT
 341 A V W Q C K H H E W G K H Y C N H N E D
 1021 GCGGTCTGGCAATGTAAACACCATGAATGGGGAAAGCATTATTGCAATCACAATGAAGAT
 361 A G V T C S D G S D L E L R L R G G G S
 1081 GCTGGCGTAACATGTTCTGATGGATCAGATCTGGAGCTAAGACTTAGAGGTGGAGGCAGC
 381 R C A G T V E V E I Q R L L G K V C D R
 1141 CGCTGTGCTGGGACAGTTGAGGTGGAGATTAGAGACTGTTAGGGAAGGTGTGTGACAGA
 401 G W G L K E A D V V C R Q L G C G S A L
 1201 GGCTGGGGACTGAAAGAAGCTGATGTGTTTGCAGGCAGCTGGGATGTGGATCTGCACTC
 421 K T S Y Q V Y S K I Q A T N M W L F L S
 1261 AAAACATCCTATCAAGTATACTCCAAAATCCAGGCAACAAACATGTGGCTGTTTCTAAGT
 441 S C N G N E T S L W D C K N W Q W G G L
 1321 AGCTGTAACGGAAATGAACTTCTCTTGGGACTGCAAGAACTGGCAATGGGGTGGACTT
 461 T C D H Y E E A K I T C S A H R E P R L
 1381 ACCTGTGATCACTATGAAGAAGCCAAAATTACCTGCTCAGCCACAGGGAACCCAGACTG
 481 V G G D I P C S G R V E V K H G D T W G
 1441 GTTGGAGGAGACATTCCCTGTTCTGGACGCGTTGAAGTGAAGCATGGTGACACATGGGGC
 501 S V C D S D F S L E A A S V L C R E L Q
 1501 TCCGTCTGTGATTCCGATTTCTCTCTGGAAGCTGCCAGCGTTCTATGCAGGGAATTACAG
 521 C G T V V S I L G G A H F G E G N G Q I
 1561 TGTGGCACAGTCGTCTCTATCCTGGGGGGAGCTCACTTTGGAGAGGGAAATGGACAGATC
 541 W T E E F Q C E G H E S H L S L C P V A
 1621 TGGACTGAAGAATTCCAGTGTGAGGGACATGAGTCCCATCTTTCACTCTGCCCAGTAGCA
 561 P R P E G T C S H S R D V G V V C S R Y
 1681 CCCCAGCCAGAAAGGAACCTGTAGCCACAGCAGGGATGTTGGAGTAGTCTGCTCAAGATAC
 581 T E I R L V N G K T P C E G R V E L K T
 1741 ACAGAAATTCGCTTGGTGAATGGCAAGACCCCATGTGAGGGCAGAGTGGAGCTCAAAACG
 601 L N A W G S L C N S H W D I E D A H V L
 1801 CTTAATGCCTGGGGATCCCTCTGCAACTCTCACTGGGACATAGAAGATGCCACGTTCTT
 621 C Q Q L K C G V A L S T P G G A H F G K
 1861 TGCCAACAACCTAAATGTGGAGTTGCCCTTTCTACCCAGGAGGAGCACATTTTGGAAAA
 641 G N G Q V W R H M F H C T G T E Q H M G
 1921 GGAAATGGTCAGGTCTGGAGGCATATGTTTCACTGCACTGGGACTGAGCAGCACATGGGA
 661 D C P V T A L G A S L C P S G Q V A S V
 1981 GATTGTCCTGTAAGTCTGCTGGGTGCTTCACTATGTCCTTACAGGGCAAGTGGCCTCTGTA
 681 I C S G N Q S Q T L S S C N S S S L G P
 2041 ATTTGCTCAGGAAACCAGTCCCAAACACTGTCCTCGTGCAATTCATCATCTCTGGGCCCA
 701 T R P T I P E E S A V A C I E S G Q L R
 2101 ACAAGGCCTACCATTCCAGAAGAAAGTGCTGTGGCCTGCATAGAGAGTGGTCAACTTCGC
 721 L V N G G G R C A G R V E I Y H E G S W
 2161 TTGGTAAATGGAGGAGGTCGCTGTGCTGGGAGAGTAGAGATTTATCATGAGGGCTCCTGG
 741 G T I C D D S W D L S D A H V V C R Q L
 2221 GGCACCATCTGTGATGACAGCTGGGACCTGAGCGATGCCACGTGGTGTGCAGACAGCTG
 761 G C G E A I N A T G S A H F G E G T G P
 2281 GGCTGTGAGAGGCCATTAATGCCACTGGTTCTGCTCATTTTGGAGAAGGAACAGGGCCC
 781 I W L D E M K C N G K E S R I W Q C H S

2341 ATCTGGCTGGATGAGATGAAATGCAATGGAAAAGAATCCCGCATTGTCAGTGCCATTCA 801 H G W G Q Q N C R H K E D A G V I C S E 2401 CATGGCTGGGGGAGCAAACTGCAGGCACAAGGAGGATGCAGGAGTTATCTGCTCAGAG 821 F M S L R L T S E A S R E A C A G R L E 2461 TTCATGTCTCTGAGACTGACCACTGAAGCCAGCAGAGAGGCCTGTGCAGGGCGTCTAGAA 841 V F Y N G A W G S V G R S N M S E T T V 2521 GTTTTTTACAACGGAGCTTGGGGCAGTGTTGGCAGGAGTAACATGTCTGAAACCACTGTG 861 G V V C R Q L G C A D K G K I N S A S L 2581 GGTGTAGTGTGCAGGCAGCTGGGCTGTGCAGACAAAGGGAAAATCAACTCTGCATCTTTA 881 D K A M S I P M W V D N V Q C P K G P D 2641 GACAAGGCCATGTCCATTCCCATGTGGGTGGACAATGTTCACTGTCCAAAAGGACCTGAC 901 T L W Q C P S S P W E K R L A R P S E E 2701 ACGCTGTGGCAGTGCCCATCATCTCCATGGGAGAAGAGACTGGCCAGGCCCTCGGAGGAG 921 T W I T C D N K M R L Q E G P T S C S G 2761 ACCTGGATCACATGTGACAACAAGATGAGACTACAAGAAGGACCCACTTCCTGTTCTGGA 941 R V E I W H G G S W G T V C D D S W D L 2821 CGTGTGGAGATCTGGCACGGAGGTTCTGGGGGACAGTGTGTGATGACTCCTGGGACTTG 961 N D A Q V V C Q Q L G C G P A L K A F K 2881 AACGATGCTCAGGTGGTGTGTCAACAACCTGGCTGTGGTCCAGCTTTGAAAGCATTCAAA 981 E A E F G Q G T G P I W L N E V K C K G 2941 GAAGCAGAGTTTGGTCAGGGGACTGGACCCATATGGCTCAATGAAGTGAAGTGCAAAGGG 1001 N E S S L W D C P A R R W G H S E C G H 3001 AATGAGTCTTCCTTGTGGGATTGTCTGCCAGACGCTGGGGCCACAGTGAGTGTGGACAC 1021 K E D A A V N C T D I S T R K T P Q K A 3061 AAGGAAGACGCTGCGGTGAATTGCACAGATATTTCAACGCGCAAACCCACAAAAAGCC 1041 T T V S S R G E N L V H Q I Q Y R E M N 3121 ACAACGGTTTCCTCAAGAGGAGAGAACTTAGTCCACCAAATTCATACCGGGAGATGAAT 1061 S C L N A D D L N L M N S S G G H S E A 3181 TCTTGCTGAATGCAGATGATCTGAACCTAATGAATTCTCAGGAGGCCATTCTGAGGCA 1081 H * K G K W E F I T Q 3241 CACTGAAAAGGAAAATGGGAATTTATAACCCAG	
1 MSKLRLVLE DSGSADVRRH FVNLSPFTIA VVLLLRACFV TSSLGGTTKE LRLVDGENKC 61 SGRVEVKIQE EWGTVCNNGW SMEAVSVICN QLGCPATAKA TGWANSSAGS GRIWMDHVSC 121 RGNESALWDC KHDGWGKHSN CTHQODAGVT CSDGSDLEMR LTNGGNMCSG RIEIKFQGW 181 GTVCDDNFNI NHASVVKQL ECGSAVSFSG SANFGEESGP IWFDDLICNG NESALWNCKH 241 QGWGKHNCBH AEDAGVICSK GADLSLRLVD GVTECSGRLE VRFQGEWGTI CDDGWDSHDA 301 AVACKQLGCP TAITAIGRVN ASEGFGHIWL DSVSCQGHEP AVWQCKHHEW GKHYCNHNE 361 AGVTCSDGSD LELRLRGGS RCAGTVEVEI QRLGKVCDR GWGLKEADV CRQLGCGSAL 421 KTSYQVYSKI QATNMWLFSL SCNGNETSLW DCKNWQWGL TCDHYEEAKI TCSAHREPL 481 VGGDIPCSGR VEVKHGDTWG SVCDSDFSLE AASVLCRELQ CGTVVSILGG AHFGEGNGQI 541 WTEEFQCEGH ESHLSLCPVA PRPEGTCSHS RDVGVCVSRV TEIRLVNGKT PCEGRVELKT 601 LNAWGLCNS HWDIEDAHLV CQQLKCGVAL STPGGAHFGK GNGQVWRHMF HCTGTQHM 661 DCPVTALGAS LCPGQVASV ICSGNQSQTLL SSCNSSSLGP TRPTIPEESA VACIESGQLR 721 LVNGGGRCAG RVEIYHEGSW GTICDDSWDL SDAHVVCRQL GCGEAINATG SAHFGGTGP 781 IWLDEMKNCG KESRIWQCHS HGWGQONCRH KEDAGVICSE FMSLRLTSEA SREACAGRL 841 VFYNGAWGSV GRSNMSETTV GVVCRLGCA DKGKINSASL DKAMSIPMWV DNVQCPKGP 901 TLWQCPSSPW EKRLARPSEE TWITCDNKM LQEGPTSCSG RVEIWHGGSW GTVCDDSWDL 961 NDAQVVCQL GCGPALKAFK EAEFGQGTGP IWLNEVKCKG NESSLWDCA RRGHSECGH 1021 KEDAVNCTD ISTRKTPQKA TTVSSRGENL VHQIYREMN SCLNADDLNL MNSSGGHSEA 1081 H	SEQ ID NO:38
1 M S K L R M V L L E D S G S A D V R R H 1 ATGAGCAAACCaGAATGGTGCTACTTGAAGACTCTGGATCTGCTGACGTGAGAAGACAT 21 F V N L S P F T I A V V L L L R A C F V 61 TTTGTCAACTTGAGTCCCTTCACTATTGCTGTGGTCTTACTTCTCCGTGCCTGTTTTGTC 41 T S S L G G T T K E L R L V D G E N K C 121 ACCAGTTCTCTTGGAGGAACAACCAAGGAGCTGAGGCTAGTGGATGGTGAAAACAAGTGT 61 S G R V E V K I Q E E W G T V C N N G W 181 AGTGGGAGAGTGGAAGTGAAAATCCAGGAGGAGTGGGGAACGGTGTGTAATAATGGCTGG 81 S M E A V S V I C N Q L G C P T A I K A 241 AGCATGGAAGCAGTCTCTGTGATTTGTAACCAGCTGGGATGTCCAACCTGCTATCAAAGCC	SEQ ID NO:39 and 40

101 T G W A N S S A G S G R I W M D H V S C
 301 ACTGGATGGGCTAATTCCAGTGCAGGTTCTGGACGCATTGATGGATCATGTTTCTTGT
 121 R G N E S A L W D C K H D G W G K H S N
 361 CGTGGGAATGAGTCAGCTCTTTGGGACTGCAAACATGATGGATGGGGAAAGCATAGTAAC
 141 C T H Q Q D A G V T C S D G S D L E M R
 421 TGTACTCACCAACAAGATGCTGGAGTGACTTGCTCAGATGGATCCGATTTGGAAATGAGG
 161 L T N G G N M C S G R I E I K F Q G Q W
 481 CTGACGAATGGAGGGAATATGTGTTCTGGAAGAATAGAGATCAAATTCCAAGGACAGTGG
 181 G T V C D D N F N V N H A S V V C K Q L
 541 GGAACAGTGTGTGATGATAACTTCAACGTCAATCATGCATCTGTGGTTTGTAAACAACTT
 201 E C G S A V S F S G S A N F G E G S G P
 601 GAATGTGGAAGTGCTGTCAGTTTCTCTGGTTCAGCTAATTTTGGAGAAGGCTCTGGACCA
 221 I W F D D L I C N G N E S A L W N C K H
 661 ATCTGGTTTGTATGATCTTATATGCAACGGAAATGAGTCAGCTCTCTGGAAGTGCAAACAT
 241 Q G W G K H N C D H A E D A G V I C S K
 721 CAAGGATGGGGAAAGCATAACTGTGATCATGCTGAGGATGCTGGAGTGATTGCTCAAAG
 261 G A D L S L R L V D G V T E C S G R L E
 781 GGAGCAGATCTGAGCCTGAGACTGGTAGATGGAGTCACTGAATGTTTCAGGAAGATTAGAA
 281 V R F Q G E W G T I C D D G W D S H D A
 841 GTGAGATTCCAAGGAGAATGGGGGACAAATATGTGATGACGGCTGGGACAGTCATGATGCT
 301 A V A C K Q L G C P T A I T A I G R V N
 901 GCTGTGGCATGCAAGCAACTGGGATGTCCAAGTCTATCACCGCCATTGGTTCGAGTTAAC
 321 A S E G F G H I W L D S V S C Q G H E P
 961 GCCAGTGAGGGATTGAGACACATCTGGCTTGACAGTGTCTTGTCCAGGGACATGAACCT
 341 A V W Q C K H H E W G K H Y C N H N E D
 1021 GCGGTCTGGCAATGTAAACACCATGAATGGGGAAAGCATTATTGCAATCACAATGAAGaT
 361 A G V T C S D G S D L E L R L R G G G S
 1081 GCTGGCGTAACATGTTCTGATGGATCAGATCTGGAGCTAAGACTTAGAGGTGGAGGCAGC
 381 R C A G T V E V E I Q R L L G K V C D R
 1141 CGCTGTGCTGGGACAGTTGAGGTGGAGATtCAGAGACTGTTAGGGAAGGTGTGTGACAGA
 401 G W G L K E A D V V C R Q L G C G S A L
 1201 GGCTGGGGACTGAAAGAAGCTGATGTGGTTTGCAGGCAGCTGGGATGTGGATCTGCACTC
 421 K T S Y Q V Y S K I Q A T N M W L F L S
 1261 AAAACATCCTATCAAGTATACTCCAAAATCCAGGCAACAAACATGTGGCTGTTTCTAAGT
 441 S C N G N E T S L W D C K N W Q W G G L
 1321 AGCTGTAACGGAAATGAAACTTCTCTTTGGGACTGCAAGAACTGGCAATGGGGTGGACTT
 461 T C D H Y E E A K I T C S A H R E P R L
 1381 ACCTGTGATCACTATGAAGAAGCCAAAATTACCTGCTCAGCCCACAGGGAACCCAGACTG
 481 V G G D I P C S G R V E V K H G D T W G
 1441 GTTGGAGGAGACATTCCCTGTTCTGGACGCGTTGAAGTGAAGCATGGTGACACATGGGGC
 501 S V C D S D F S L E A A S V L C R E L Q
 1501 TCCGTCTGTGATTTCGATTCTCTCTGGAAGCTGCCAGCGTTCTATGCAGGGAATTACAG
 521 C G T V V S I L G G A H F G E G N G Q I
 1561 TGTGGCACAGTCGTCTCTATCCTGGGGGGAGCTCACTTTGGAGAGGGAAATGGACAGATC
 541 W A E E F Q C E G H E S H L S L C P V A
 1621 TGGGCTGAAGAATTCCAGTGTGAGGGACATGAGTCCCATCTTTCACCTCTGCCAGTAGCA
 561 P R P E G T C S H S R D V G V V C S R Y
 1681 CCCC GCCCAGAAGGAACTTGTAGCCACAGCAGGGATGTTGGAGTAGTCTGCTCAAGATAC
 581 T E I R L V N G K T P C E G R V E L K T
 1741 ACAGAAATTCGCTTGGTGAATGGCAAGACCCCATGTGAGGGCAGAGTGGAGCTCAAAACG
 601 L N A W G S L C N S H W D I E D A H V L
 1801 CTTAATGCCTGGGGATCCCTCTGCAACTCTCACTGGGACATAGAAGATGCCACGTTCTT
 621 C Q Q L K C G V A L S T P G G A H F G K
 1861 TGCCAACAACCTTAAATGTGGAGTTGCCCTTTCTACCCAGGAGGAGCACATTTTGGAAAA
 641 G N G Q V W R H M F H C T G T E Q H M G

1921 GGAAATGGTCAGGTCTGGAGGCATATGTTTCACTGCACTGGGACTGAGCAGCACATGGGA	
661 D C P V T A L G A S L C P S G Q V A S V	
1981 GATTGTCCTGTAAGTCTGCTGGGTGCTTCACTATGTCCTTCAGGGCAAGTGGCCTCTGTA	
681 I C S G N Q S Q T L S S C N S S S L G P	
2041 ATTTGCTCAGGAAACCAGTCCCAAACACTGTCTCTGCAATTCATCATCTCTGGGCCCA	
701 T R P T I P E E S A V A C I E S G Q L R	
2101 ACAAGGCCTACCATTCCAGAAGAAAGTGTGTGGCCTGCATAGAGAGTGGTCAACTTCGC	
721 L V N G G G R C A G R V E I Y H E G S W	
2161 TTGGTAAATGGAGGAGGTGCTGTGCTGGGAGAGTAGAGATTTATCATGAGGGCTCCTGG	
741 G T I C D D S W D L S D A H V V C R Q L	
2221 GGCACCATCTGTGATGACAGCTGGGACCTGAGCGATGCCACGTGGTGTGCAGACAGCTG	
761 G C G E A I N A T G S A H F G E G T G P	
2281 GGCTGTGGAGAGGCCATTAATGCCACTGGTTCTGCTCATTTTGGAGAAGGAACAGGGCCC	
781 I W L D E M K C N G K E S R I W Q C H S	
2341 ATCTGGCTGGATGAGATGAAATGCAATGGAAAAGAATCCCGTATTTGGCAGTGCCATTCA	
801 H G W G Q Q N C R H K E D A G V I C S E	
2401 CATGGCTGGGGGCAGCAAACTGCAGGCACAAGGAGGATGCAGGAGTTATCTGCTCAGAG	
821 F M S L R L T S E A S R E A C A G R L E	
2461 TTCATGTCTCTGAGACTGACCAGTGAAGCCAGCAGAGAGGCCTGTGCAGGGCGTCTAGAA	
841 V F Y N G A W G S V G R S N M S E T T V	
2521 GTTTTTTACAACGGAGCTTGGGGCAGTGTGGCAGGAGTAACATGTCTGAAACCACTGTG	
861 G V V C R Q L G C A D K G K I N S A S L	
2581 GGTGTGGTGTGCAGGCAGCTGGGCTGTGCAGACAAAGGGAAAATCAACTCTGCATCTTTA	
881 D K A M S I P M W V D N V Q C P K G P D	
2641 GACAAGGCCATGTCCATTCCCATGTGGGTGGACAATGTTCACTGTCCAAAAGGACCTGAC	
901 T L W Q C P S S P W E K R L A R P S E E	
2701 ACGCTGTGGCAGTGCCCATCATCTCCATGGGAGAAGAGACTGGCCAGGCCCTCGGAGGAG	
921 T W I T C D N K M R L Q E G P T S C S G	
2761 ACCTGGATCACATGTGACAACAAGATGAGACTACAAGAAGGACCCACTTCCTGTTCTGGA	
941 R V E I W H G G S W G T V C D D S W D L	
2821 CGTGTGGAGATCTGGCACGGAGGTTCTGGGGGACAGTGTGTGATGACTCCTGGGACTTG	
961 N D A Q V V C Q Q L G C G P A L K A F K	
2881 AACGATGCTCAGGTGGTGTGTCAACAACCTGGCTGTGGTCCAGCTTTGAAAGCATTCAAA	
981 E A E F G Q G T G P I W L N E V K C K G	
2941 GAAGCAGAGTTTGGTCAGGGGACTGGACCCATATGGCTCAATGAAGTGAAGTGCAAAGGG	
1001 N E S S L W D C P A R R W G H S E C G H	
3001 AATGAGTCTTCTTGTGGGATTGTCTGCCAGACGCTGGGGCCACAGTGAGTGTGGACAC	
1021 K E D A A V N C T A Q K I S T H K T P Q	
3061 AAGGAAGACGCTGCAGTGAATTGCACAGCACAAAAAATTTCAACGCACAAAACCCACAA	
1041 K A T T V S S R G E N L V H Q I Q Y R E	
3121 AAAGCCACAACAGTTTCTCAAGAGGAGAGAACTTAGTCCACCAAATTCATACCGGGAG	
1061 M N S C L N A D D L D L M N S S G G H S	
3181 ATGAATTCTTGCTGAATGCAGATGATCTGGACCTAATGAATTCTCAGGAGGCCATTCT	
1081 E A H * K G K W E F I T Q	
3241 GAGGCACACTGAAAAGGAAAATGGGAATTTATAACCCAG	
1 MSKLRMVLE DSGSADVRRH FVNLSPTTIA VVLLLRACFV TSSLGGTTKE LRLVDGENKC	SEQ ID NO:40
61 SGRVEVKIQE EWGTVCNNGW SMEAVSVICN QLGCPTAICA TGWANSSAGS GRIWMDHVSC	
121 RGNESALWDC KHDGWGKHSN CTHQQDAGVT CSDGSDLEMR LTNGGNMCSG RIEIKFQGQW	
181 GTVCDDNFNV NHASVVCKQL ECGSAVSFSG SANFGECSGP IWFDDLICNG NESALWNCKH	
241 QGWGKHNCBH AEDAGVICK GADLSLRLVD GVTECSGRLE VRFQGEWGTI CDDGWDSDHA	
301 AVACKQLGCP TAITAIGRVN ASEGFGHIWL DSVSCQHEP AVWQCKHHEW GKHYCNHNE	
361 AGVTCSDGSD LELRLRGSGS RCAGTVEVEI QRLLGKVCDR GWGLKEADV CRQLGCGSAL	
421 KTSYQVYSKI QATNMWLFSL SCNGNETSLW DCKNWQWGGT TCDHYEEAKI TCSAHREPR	
481 VGGDIPCSGR VEVKHGDTWG SVCDSDFSLE AASVLCRELQ CGTVVSILGG AHFGEENGQI	
541 WAEFQCEGH ESHLSLCPVA PRPEGTCSHS RDVGVCVCSRY TEIRLVNGKT PCEGRVELKT	
601 LNAWGLCNS HWDIEDAHLV CQQLKCGVAL STPGGAHFGK GNGQVWRHMF HCTGTEQHM	
661 DCPVTALGAS LCPSGQVASV ICSGNQSQTLL SSCNSSSLGP TRPTIPEESA VACIESGQLR	
721 LVNGGGRCAG RVEIYHEGSW GTICDDSWDL SDAHVVCRQL GCGEAINATG SAHFGEETGP	
781 IWLDEMKNCG KESRIWQCHS HGWGQONCRH KEDAGVICSE FMSLRILTSEA SREACAGRLE	
841 VFYNGAWGSV GRSNMSETTV GVVCRLGCA DKGKINSASL DKAMSIPMWV DNVQCPKGP	

901 TLWQCPSSPW EKRLARPSEE TWITCDNKMRLQEGPTSCSG RVEIWHGGSW GTVCDDSWDL 961 NDAQVVCQQL GCGPALKAFK EAEFGQGTGP IWLNEVKCKG NESSLWDCPA RRWGHSECGH 1021 KEDAAVNCTA QKISTHKTPQ KATTVSSRGE NLVHQIQYRE MNSCLNADDL DLMNSSGGHS 1081 EAH	
1 M S K L R M V L L E D S G S A D V R R H 1 ATGAGCAAACCTCAGAATGGTGCTACTTGAAGACTCTGGATCTGCTGACGTCAGAAGACAT 21 F V N L S P F T I A V V L L L R A C F V 61 TTTGTCAACTTGAGTCCCTTCACTATTGCTGTGGTCTTACTTCTCCGTGCCTGTTTTGTC 41 T S S L G G T T K E L R L V D G E N K C 121 ACCAGTTCTCTTGGAGGAACAACCAAGGAGCTGAGGCTAGTGGATGGTGAAAACAAGTGT 61 S G R V E V K I Q E E W G T V C N N G W 181 AGTGGGAGAGTGGAAGTGAAAATCCAGGAGGAGTGGGGAACGGTGTGTAATAATGGCTGG 81 S M E A V S V I C N Q L G C P T A I K A 241 AGCATGGAAGCAGTCTCTGTGATTGTGAACCAGCTGGGATGTCCAACCTGCTATCAAAGCC 101 T G W A N S S A G S G R I W M D H V S C 301 ACTGGATGGGCTAATTCCAGTGCAGGTTCTGGACGCATTTGGATGGATCATGTTTCTTGT 121 R G N E S A L W D C K H D G W G K H S N 361 CGTGGGAATGAGTCAGCTCTTTGGGACTGCAACATGATGGATGGGGAAGCATAGTAAC 141 C T H Q Q D A G V T C S D G S D L E M R 421 TGTACTCACCACAAGATGCTGGAGTGAAGTGTCTCAGATGGATCCGATTTGGAAATGAGG 161 L T N G G N M C S G R I E I K F Q G Q W 481 CTGACGAATGGAGGGAATATGTGTTCTGGAAGAATAGAGATCAAATCCAAGGACAGTGG 181 G T V C D D N F N I N H A S V V C K Q L 541 GGAACAGTGTGTGATGATAACTTCAACATCAATCATGCATCTGTGGTTTGTAACAACCTT 201 E C G S A V S F S G S A N F G E G S G P 601 GAATGTGGAAGTGTGTCAGTTTCTCTGGTTCTAGCTAATTTTGGAGAAGGCTCTGGACCA 221 I W F D D L I C N G N E S A L W N C K H 661 ATCTGGTTTGATGATCTTATATGCAACGGAAATGAGTCAGCTCTCTGGAACCTGCAACAT 241 Q G W G K H N C D H A E D A G V I C S K 721 CAAGGATGGGGAAGCATAACTGTGATCATGCTGAGGATGCTGGAGTGATTGCTCAAAG 261 G A D L S L R L V D G V T E C S G R L E 781 GGAGCAGATCTGAGCCTGAGACTGGTAGATGGAGTCACTGAATGTTTCAAGGAAGATTAGAA 281 V R F Q G E W G T I C D D G W D S H D A 841 GTGAGATTCCAAGGAGAATGGGGGACAATATGTGATGACGGCTGGGACAGTCATGATGCT 301 A V A C K Q L G C P T A I T A I G R V N 901 GCTGTGGCATGCAAGCAACTGGGATGTCCAACCTGCTATCACCGCCATTGGTTCGAGTTAAC 321 A S E G F G H I W L D S V S C Q G H E P 961 GCCAGTGAGGGATTGGACACATCTGGCTTGACAGTGTCTTCTTGCCAGGGACATGAACCT 341 A V W Q C K H H E W G K H Y C N H N E D 1021 GCGGTCTGGCAATGTAAACACCATGAATGGGGAAGCATTATTGCAATCACAATGAAGAT 361 A G V T C S D G S D L E L R L R G G G S 1081 GCTGGCGTAACATGTTCTGATGGATCAGATCTGGAGCTAAGACTTAGAGGTGGAGGCAGC 381 R C A G T V E V E I Q R L L G K V C D R 1141 CGCTGTGCTGGGACAGTTGAGGTGGAGATTGAGAGACTGTTAGGGAAGGTGTGtGACAGA 401 G W G L K E A D V V C R Q L G C G S A L 1201 GGCTGGGGACTGAAAGAAGCTGATGTGGTTTGCAGGCAGCTGGGATGTGGATCTGCACTC 421 K T S Y Q V Y S K I Q A T N M W L F L S 1261 AAAACATCCTATCAAGTATACTCCAAAATCCAGGCAACAAACATGTGGCTGTTTCTAAGT 441 S C N G N E T S L W D C K N W Q W G G L 1321 AGCTGTAACGGAAATGAAACTTCTCTTTGGGACTGCAAGAACTGGCAATGGGGTGGACTT 461 T C D H Y E E A K I T C S A H R E P R L 1381 ACCTGTGATCACTATGAAGAAGCCAAAATTACCTGCTCAGCCCACAGGGAACCCAGACTG 481 V G G D I P C S G R V E V K H G D T W G 1441 GTTGGAGGAGACATTCCCTGTTCTGGACGCGTTGAAGTGAAGCATGGTGACACATGGGGC 501 S V C D S D F S L E A A S V L C R E L Q	SEQ ID NO: 41 and 42

1501 TCCGTCTGTGATTTCGGATTTCTCTCTGGAAGCTGCCAGCGTTCTATGCAGGGAATTACAG
 521 C G T V V S I L G G A H F G E G N G Q I
 1561 TGTGGCACAGTCGTCTCTATCCTGGGGGGAGCTCACTTTGGAGAGGGAAATGGACAGATC
 541 W A E E F Q C E G H E S H L S L C P V A
 1621 TGGGCTGAAGAATTCCAGTGTGAGGGACATGAGTCCCATCTTTCACTCTGCCCAGTAGCA
 561 P R P E G T C S H S R D V G V V C S R Y
 1681 CCCC GCCAGAAGGAACCTTGTAGCCACAGCAGGGATGTTGGAGTAGTCTGCTCAAGATAC
 581 T E I R L V N G K T P C E G R V E L K T
 1741 ACAGAAATTCGCTTGGTGAATGGCAAGACCCCATGTGAGGGCAGAGTGGAGCTCAAAACG
 601 L N A W G S L C N S H W D I E D A H V L
 1801 CTTAATGCCTGGGGATCCCTCTGCAACTCTCACTGGGACATAGAAGATGCCACGTTCTT
 621 C Q Q L K C G V A L S T P G G A H F G K
 1861 TGCCAACAACCTTAAATGTGGAGTTGCCCTTTCTACCCAGGAGGAGCACATTTTGGAAAA
 641 G N G Q V W R H M F H C T G T E Q H M G
 1921 GGAAATGGTCAGGTCTGGAGGCATATGTTTCACTGCACTGGGACTGAGCAGCACATGGGA
 661 D C P V T A L G A S L C P S G Q V A S V
 1981 GATTGTCCTGTAAGTCTGCTGGGTGCTTCACTATGTCCTTCAGGGCAAGTGGCCTCTGTA
 681 I C S G N Q S Q T L S S C N S S S L G P
 2041 ATTTGCTCAGGAAACAGTCCCAAACACTGTCTCGTGCAATTCATCATCTCTGGGCCCA
 701 T R P T I P E E S A V A C I E S G Q L R
 2101 ACAAGGCCTACCATTCCAGAAGAAAGTGTGTGGCCTGCATAGAGAGTGGTCAACTTCGC
 721 L V N G G G R C A G R V E I Y H E G S W
 2161 TTGGTAAATGGAGGAGGTGCTGTGCTGGGAGAGTAGAGATTTATCATGAGGGCTCCTGG
 741 G T I C D D S W D L S D A H V V C R Q L
 2221 GGCACCATCTGTGATGACAGCTGGGACCTGAGCGATGCCACGTTGGTGTGCAGACAGCTG
 761 G C G E A I N A T G S A H F G E G T G P
 2281 GGCTGTGGAGAGGCCATTAATGCCACTGGTTCTGCTCATTTTGGAGAAGGAACAGGGCCC
 781 I W L D E M K C N G K E S R I W Q C H S
 2341 ATCTGGCTGGATGAGATGAAATGCAATGGAAAAGAATCCCGTATTTGGCAGTGCCATTCA
 801 H G W G Q Q N C R H K E D A G V I C S E
 2401 CATGGCTGGGGGCAGCAAACTGCAGGCACAAGGAGGATGCAGGAGTTATCTGCTCAGAG
 821 F M S L R L T S E A S R E A C A G R L E
 2461 TTCATGTCTCTGAGACTGACCAGTGAAGCCAGCAGAGAGGCCTGTGCAGGGCGTCTAGAA
 841 V F Y N G A W G S V G R S N M S E T T V
 2521 GTTTTTTACAACGGAGCTTGGGGCAGTGTGGCAGGAGTAACATGTCTGAAACCACTGTG
 861 G V V C R Q L G C A D K G K I N S A S L
 2581 GGTGTGGTGTGCAGGCAGCTGGGCTGTGCAGACAAAGGGAAAATCAACTCTGCATCTTTA
 881 D K A M S I P M W V D N V Q C P K G P D
 2641 GACAAGGCCATGTCCATTCCCATGTGGGTGGACAATGTTCACTGTCCAAAAGGACCTGAC
 901 T L W Q C P S S P W E K R L A R P S E E
 2701 ACGCTGTGGCAGTGCCCATCATCTCCATGGGAGAAGAGACTGGCCAGGCCCTCGGAGGAG
 921 T W I T C D N K M R L Q E G P T S C S G
 2761 ACCTGGATCACATGTGACAACAAGATGAGACTACAAGAAGGACCCACTTCCTGTTCTGGA
 941 R V E I W H G G S W G T V C D D S W D L
 2821 CGTGTGGAGATCTGGCACGGAGGTTCTGGGGGACAGTGTGTGATGACTCCTGGGACTTG
 961 N D A Q V V C Q Q L G C G P A L K A F K
 2881 AACGATGCTCAGGTGGTGTGTCAACAACCTGGCTGTGGTCCAGCTTTGAAAGCATTCAAA
 981 E A E F G Q G T G P I W L N E V K C K G
 2941 GAAGCAGAGTTTGGTCAGGGGACTGGACCCATATGGCTCAATGAAGTGAAGTGCAAAGGG
 1001 N E S S L W D C P A R R W G H S E C G H
 3001 AATGAGTCTTCCTTGTGGGATTGTCTCTGCCAGACGCTGGGGCCACAGTGAGTGTGGACAC
 1021 K E D A A V N C T A Q K I S T H K T P Q
 3061 AAGGAAGACGCTGCAGTGAATTGCACAGCACAAAAATTTCAACGCACAAAACCCACAA
 1041 K A T T G R S F L I A F G I L G V V L L
 3121 AAAGCCACAACAGGTCTGGTCATTCTTATTGCATTCTGGAATCCTTGGAGTTGTTCTCTTG

1061 A I F V A L F L T Q K R R Q R Q R L T V 3181 GCCATTTTCGTCGCATTATTCTTGACTCAAAGCGAAGACAGAGACAGCGGCTTACAGTT 1081 S S R G E N L V H Q I Q Y R E M N S C L 3241 TCCTCAAGAGGAGAGAACTTAGTCCACCAAATTCAATACCGGGAGATGAATTCTTGCCTG 1101 N A D D L D L M N S S G G H S E A H 3301 AATGCAGATGATCTGGACCTAATGAATTCCTCAGGAGGCCATTCTGAGGCACAC	
1 MSKLRLMVLLE DSGSADVRRH FVNLSPTTIA VVLLLRACFV TSSLGGTTKE LRLVDGENKC 61 SGRVEVKIQE EWGTVCNNGW SMEAVSVICN QLGCPATAKA TGWANSAGS GRIWMDHVSC 121 RGNESALWDC KHDGWGKHSN CTHQQDAGVT CSDGSDLEMR LTNGGNMCSG RIEIKFQGQW 181 GTVCDDNFNI NHASVVCQL ECGSAVSFSG SANFGEESGP IWFDDLICNG NESALWNCKH 241 QGWGKHNCNDH AEDAGVICSK GADLSLRLVD GVTECSGRLE VRFQGEWGTI CDDGWDSHDA 301 AVACKQLGCP TAITAIGRVN ASEGFGHIWL DSVSCQGHEP AVWQCKHHEW GKHVCNHNED 361 AGVTCSDGSD LELRLRGGGS RCAGTVEVEI QRLLGKVCDR GWGLKEADV CRQLGCGSAL 421 KTSYQVYSKI QATNMWLFSL SCNGNETSLW DCKNWQWGGT TCDHYEEAKI TCSAHREPR 481 VGGDIPCSGR VEVKHGDTWG SVCDSDFSLE AASVLCRELQ CGTVVSILGG AHFGEENGQI 541 WAEFQCEGH ESHLSLCPVA PRPEGTCSHS RDVGVCSTRY TEIRLVNGKT PCEGRVELKT 601 LNAWGSCLNS HWDIEDAHLV CQQLKCGVAL STPGGAHFGK GNGQVWRHMF HCTGTEQHMG 661 DCPVTALGAS LCPSGQVASV ICSGNQSQTL SSCNSSSLGP TRPTIPEESA VACIESGQLR 721 LVNGGGRCAG RVEIYHEGSW GTICDDSDWL SDAHVVCRQL GCGEAINATG SAHFGEETGP 781 IWLDEMKCNG KESRIWQCHS HGWGQONCRH KEDAGVICSE FMSLRILTSEA SREACAGRL 841 VFYNGAWGSV GRSNMSETTV GVVCRLGCA DKGKINSASL DKAMSIPMWV DNVQCPKGP 901 TLWQCPSSPW EKRLARPSEE TWITCDNMR LQEGPTSCSG RVEIWHGGSW GTVCDDSWDL 961 NDAQVVCQL GCGPALKAFK EAEFGQGTGP IWLNEVKCKG NESSLWDCA RRWGHSECGH 1021 KEDAAVNCTA QKISTHKTPQ KATTGRSFLI AFGILGVVLL AIFVALFLTQ KRRQRQLTV 1081 SSRGENLVHQ IQYREMNCL NADDLDLMNS SGGHSEAH	SEQ ID NO: 42
1 M S K L R M V L L E D S G S A D V R R H 1 ATGAGCAAATCAGAATGGTGTCTACTTGAAGACTCTGGATCTGCTGACGTCAGAAGACAT 21 F V N L S P F T I A V V L L L R A C F V 61 TTTGTCAACTTGAGTCCCTTCACTATTGCTGTGGTCTTACTTCTCCGTGCCTGTTTGTGTC 41 T S S L G G T T K E L R L V D G E N K C 121 ACCAGTTCTCTTGAGGAACAACCAAGGAGCTGAGGCTAGTGGATGGTGAAAACAAGTGT 61 S G R V E V K I Q E E W G T V C N N G W 181 AGTGGGAGAGTGGAAGTGAAATCCAGGAGGAGTGGGGAACGGTGTGTAATAATGGCTGG 81 S M E A V S V I C N Q L G C P T A I K A 241 AGCATGGAAGCAGTCTCTGTGATTTGTAACCAGCTGGGATGTCCAAGTCTATCAAAGCC 101 T G W A N S S A G S G R I W M D H V S C 301 ACTGGATGGGCTAATTCAGTGCAGGTTCTGGACGCATTTGGATGGATCATGTTTCTTGT 121 R G N E S A L W D C K H D G W G K H S N 361 CGTGGGAATGAGTCAGCTCTTTGGGACTGCAACATGATGGATGGGGAAGCATAGTAAC 141 C T H Q Q D A G V T C S D G S D L E M R 421 TGTACTCACCACAAGATGCTGGAGTGACTTGCTCAGATGGATCCGATTTGGAATGAGG 161 L T N G G N M C S G R I E I K F Q G Q W 481 CTGACGAATGGAGGGAATATGTGTTCTGGAAGAATAGAGATCAAATCCAAGGACAGTGG 181 G T V C D D N F N I N H A S V V C K Q L 541 GGAACAGTGTGTGATGATAACTTCAACATCAATCATGCATCTGTGGTTTGTAACAACCTT 201 E C G S A V S F S G S A N F G E G S G P 601 GAATGTGGAAGTGCTGTGAGTTTCTCTGGTTCAGCTAATTTTGGAAGAAGGCTCTGGACCA 221 I W F D D L I C N G N E S A L W N C K H 661 ATCTGGTTTGATGATCTTATATGCAACGGAAATGAGTCAGCTCTCTGGAAGTCAAACAT 241 Q G W G K H N C D H A E D A G V I C S K 721 CAAGGATGGGGAAGCATAACTGTGATCATGCTGAGGATGCTGGAGTGATTTGCTCAAAG 261 G A D L S L R L V D G V T E C S G R L E 781 GGAGCAGATCTGAGCCTGAGACTGGTAGATGGAGTCACTGAATGTTTCAAGGAGATTAGAA 281 V R F Q G E W G T I C D D G W D S H D A 841 GTGAGATTCCAAGGAGAATGGGGGACAATATGTGATGACGGCTGGGACAGTCATGATGCT 301 A V A C K Q L G C P T A I T A I G R V N 901 GCTGTGGCATGCAAGCAACTGGGATGTCCAAGTCTATCACCGCCATTGGTCGAGTTAAC 321 A S E G F G H I W L D S V S C Q G H E P 961 GCCAGTGAGGGATTGACACATCTGGCTTGACAGTGTCTTCTGCCAGGGACATGAACCT	SEQ ID NO: 43 and 44

341 A V W Q C K H H E W G K H Y C N H N E D
 1021 GCGGTCTGGCAATGTAAACACCATGAATGGGGAAGCATTATTGCAATCACAATGAAGAT
 361 A G V T C S D G S D L E L R L R G G G S
 1081 GCTGGCGTAACATGTTCTGATGGATCAGATCTGGAGCTAAGACTTAGAGGTGGAGGCAGC
 381 R C A G T V E V E I Q R L L G K V C D R
 1141 CGCTGTGCTGGGACAGTTGAGGTGGAGATTGAGAGACTGTTAGGGAAGGTGTGTGACAGA
 401 G W G L K E A D V V C R Q L G C G S A L
 1201 GGCTGGGGACTGAAAGAAGCTGATGTGGTTTGCAGGCAGCTGGGATGTGGATCTGCACTC
 421 K T S Y Q V Y S K I Q A T N M W L F L S
 1261 AAAACATCCTATCAAGTATACTCCAAAATCCAGGCAACAAACATGTGGCTGTTTCTAAGT
 441 S C N G N E T S L W D C K N W Q W G G L
 1321 AGCTGTAAACGGAATGAACTTCTCTTTGGGACTGCAAGAACTGGCAATGGGGTGGACTT
 461 T C D H Y E E A K I T C S A H R E P R L
 1381 ACCTGTGATCACTATGAAGAAGCCAAAATTACCTGCTCAGCCCACAGGGAACCCAGACTG
 481 V G G D I P C S G R V E V K H G D T W G
 1441 GTTGGAGGAGACATTCCCTGTTCTGGACGCGTTGAAGTGAAGCATGGTGACACATGGGGC
 501 S V C D S D F S L E A A S V L C R E L Q
 1501 TCCGTCTGTGATTTCGGATTTCTCTCTGGAAGCTGCCAGCGTTCTATGCAGGGAATTACAG
 521 C G T V V S I L G G A H F G E G N G Q I
 1561 TGTGGCACAGTCGTCTCTATCCTGGGGGGAGCTCACTTTGGAGAGGGAAATGGACAGATC
 541 W A E E F Q C E G H E S H L S L C P V A
 1621 TGGGCTGAAGAATTCCAGTGTGAGGGACATGAGTCCCATCTTTCCTCTGCCCAGTAGCA
 561 P R P E G T C S H S R D V G V V C S R Y
 1681 CCCCAGCCAGAAAGGAAGTGTAGCCACAGCAGGGATGTTGGAGTAGTCTGCTCAAGATAC
 581 T E I R L V N G K T P C E G R V E L K T
 1741 ACAGAAATTCGCTTGGTGAATGGCAAGACCCCATGTGAGGGCAGAGTGGAGCTCAAAACG
 601 L N A W G S L C N S H W D I E D A H V L
 1801 CTTAATGCCTGGGGATCCCTCTGCAACTCTCACTGGGACATAGAAGATGCCACGTTCTT
 621 C Q Q L K C G V A L S T P G G A H F G K
 1861 TGCCAACAACCTTAAATGTGGAGTTGCCCTTTCTACCCAGGAGGAGCACATTTTGGAAAA
 641 G N G Q V W R H M F H C T G T E Q H M G
 1921 GGAAATGGTCAGGTCTGGAGGCATATGTTTCACTGCACTGGGACTGAGCAGCACATGGGA
 661 D C P V T A L G A S L C P S G Q V A S V
 1981 GATTGTCCTGTAAGTCTGCTGCTGGGTGCTTCACTATGTCCTTCAGGGCAAGTGGCCTCTGTA
 681 I C S G N Q S Q T L S S C N S S S L G P
 2041 ATTTGCTCAGGAAACCAGTCCCAAACACTGTCCTCGTGCAATTCATCATCTCTGGGCCCA
 701 T R P T I P E E S A V A C I E S G Q L R
 2101 ACAAGGCCTACCATTCCAGAAGAAAGTGCTGTGGCCTGCATAGAGAGTGGTCAACTTCGC
 721 L V N G G G R C A G R V E I Y H E G S W
 2161 TTGGTAAATGGAGGAGTCGCTGTGCTGGGAGAGTAGAGATTTATCATGAGGGCTCCTGG
 741 G T I C D D S W D L S D A H V V C R Q L
 2221 GGCACCATCTGTGATGACAGCTGGGACCTGAGCGATGCCCACGTGGTGTGACAGAGCTG
 761 G C G E A I N A T G S A H F G E G T G P
 2281 GGCTGTGGAGAGGCCATTAATGCCACTGGTTCTGCTCATTTTGGAGAAGGAACAGGGCCC
 781 I W L D E M K C N G K E S R I W Q C H S
 2341 ATCTGGCTGGATGAGATGAAATGCAATGGAAAAGAATCCCGTATTTGGCAGTGCCATTCA
 801 H G W G Q Q N C R H K E D A G V I C S E
 2401 CATGGCTGGGGGCAGCAAACTGCAGGCACAAGGAGGATGCAGGAGTTATCTGCTCAGAG
 821 F M S L R L T S E A S R E A C A G R L E
 2461 TTCATGTCTCTGAGACTGACCAGTGAAGCCAGCAGAGAGGCCTGTGCAGGGCGTCTAGAA
 841 V F Y N G A W G S V G R S N M S E T T V
 2521 GTTTTTTACAACGGAGCTTGGGGCAGTGTGGCAGGAGTAACATGTCTGAAACCACTGTG
 861 G V V C R Q L G C A D K G K I N S A S L
 2581 GGTGTGGTGTGCAGGCAGCTGGGCTGTGCAGACAAAGGGAAAATCAACTCTGCATCTTTA
 881 D K A M S I P M W V D N V Q C P K G P D

2641 GACAAGGCCATGTCCATTCCCATGTGGGTGGACAATGTTTCAGTGTCCAAAAGGACCTGAC 901 T L W Q C P S S P W E K R L A R P S E E 2701 ACGCTGTGGCAGTGCCCATCATCTCCATGGGAGAAGAGACTGGCCAGGCCCTCGGAGGAG 921 T W I T C D N K M R L Q E G P T S C S G 2761 ACCTGGATCACATGTGACAACAAGATGAGACTACAAGAAGGACCCACTTCCTGTTCTGGA 941 R V E I W H G G S W G T V C D D S W D L 2821 CGTGTGGAGATCTGGCACGGAGGTTCTGGGGGACAGTGTGTGATGACTCCTGGGACTTG 961 N D A Q V V C Q Q L G C G P A L K A F K 2881 AACGATGCTCAGGTGGTGTGTCAACAACCTGGCTGTGGTCCAGCTTTGAAAGCATTCAAA 981 E A E F G Q G T G P I W L N E V K C K G 2941 GAAGCAGAGTTTGGTCAGGGGACTGGACCCATATGGCTCAATGAAGTGAAGTGCAAAGGG 1001 N E S S L W D C P A R R W G H S E C G H 3001 AATGAGTCTTCCTTGTGGGATTGTCTGCCAGACGCTGGGGCCACAGTGAGTGTGGACAC 1021 K E D A A V N C T A Q K I S T H K T P Q 3061 AAGGAAGACGCTGCAGTGAATTGCACAGCACAAAAATTTCAACGCACAAAACCCACAA 1041 K A T T G Q S F L I A F G I L G V V L L 3121 AAAGCCACAACAGGTCAGTCATTCTTATTGCATTTCGGAATCCTTGGAGTTGTTCTCTTG 1061 A I F V A L F L T Q K R R Q R Q R L T V 3181 GCCATTTTCGTCGCATTATTCTTGACTCAAAAGCGAAGACAGAGACAGCGGCTTACAGTT 1081 S S R G E N L V H Q I Q Y R E M N S C L 3241 TCCTCAAGAGGAGAGAACTTAGTCCACCAAATTCAATACCGGGAGATGAATTCTTGCCTG 1101 N A D D L D L M N S S E N S N E S A D F 3301 AATGCAGATGATCTGGACCTAATGAATTCCTCAGAAAATTCGAATGAGTCAGCTGATTC 1121 N A A E L I S V S K F L P I S G M E K E 3361 AATGCTGCTGAACTAATTTCTGTGTCTAAATTTCTTCCTATTTCTGGAATGGAAAAGGAG 1141 A I L R H T E K E N G N L 3421 GCCATTCTGAGGCACACTGAAAAGGAAAATGGGAATTTA	
1 MSKLRMVLL DSGSADVRRH FVNLSPFTIA VVLLLRACFV TSSLGGTTKE LRLVDGENKC 61 SGRVEVKIQE EWGTVCNNGW SMEAVSVICN QLGCPATAKA TGWANSSAGS GRIWMDHVSC 121 RGNESALWDC KHDGWGKHSN CTHQQDAGVT CSDGSDLEMR LTNGGNMCSG RIEIKFQGQW 181 GTVCDDNFNI NHASVVCQQL ECGSAVSFSG SANFGEESGP IWFDDLICNG NESALWNCKH 241 QGWGKHNCNDH AEDAGVICSK GADLSLRLVD GVTECSGRLE VRFQGEWGTI CDDGWDSHDA 301 AVACKQLGCP TAITAIGRVN ASEGFQHIWL DSVSCQGHEP AVWQCKHHEW GKHYCNHNED 361 AGVTCSDGSD LELRLRGGGS RCAGTVEVEI QRLLGKVCDR GWGLKEADV V CRQLGCGSAL 421 KTSYQVYSKI QATNMWLFSL SCNGNETSLW DCKNWQWGGL TCDHYEEAKI TCSAHREPR L 481 VGGDIPCSGR VEVKHGDTWG SVCDSDFSLE AASVLCRELQ CGTVVSILGG AHFGEENGQI 541 WAEFQCEGH ESHLSLCPVA PRPEGTCSHS RDVGVCVSR Y TEIRLVNGKT PCEGRVELKT 601 LNAWGLCNS HWDIEDAHLV CQQLKCGVAL STPGGAHFGK GNGQVWRHMF HCTGTQEHMG 661 DCPVTALGAS LCPSGQVASV ICSGNQSQTL SSCNSSSLGP TRPTIPEESA VACIESGQLR 721 LVNGGGRCAG RVEIYHEGSW GTICDDSWDL SDAHVVCRQL GCGEAINATG SAHFGEETGP 781 IWLDEMKNCG KESRIWQCHS HGWGQQNCRH KEDAGVICSE FMSLRLTSEA SREACAGRL E 841 VFYNGAWGSV GRSNMSETTV GVVCRLGCA DKGKINSASL DKAMSIPMWV DNVQCPKGP D 901 TLWQCPSSPW EKRLARPSEE TWITCDNKM R LQEGPTSCSG RVEIWHGGSW GTVCDDSWDL 961 NDAQVVCQQL GCGPALKAFK EAEFGQGTGP IWLNEVKCKG NESSLWD CPA RRWGHSECGH 1021 KEDAAVNCTA QKISTHKTPQ KATTGQSFLI AFGILGVVLL AIFVALFLTQ KRRQRQLTV 1081 SSRGENLVHQ IQYREMNSCL NADDLDLMNS SENSNESADF NAAELISVSK FLPISGMEKE 1141 AILRHTEKEN GNL	SEQ ID NO: 44

Example 10 Cloning and characterization of canine CD163 from DH82 cells.

A forward primer 5'simianCD163 (SEQ ID NO: 28) (5'-

- 5 CACCGGAATGAGCAAACCTCAGAATGG-3' based on human CD163) and a reverse primer HuCD163-3'Kpn (SEQ ID NO: 29) (5'-

GCTCCGGTACCTAGTCCAGGTCTTCA

TCAAGGTATCTTA-3') were used to amplify CD163 cDNA from DH82 cells. Total cellular RNA was prepared from DH82 cells using the RNeasy kit. RT-PCR

5
10

SEQUENCE	ID NO
1 M S K L R M V P H G N S G S A D F R R C 1 ATGAGCAAACCTCAGAATGGTCCCACATGGAAACTCTGGATCTGCTGACTTTAGAAGATGT 21 F A L L C P S A V A V V S I L S T C L M 61 TTTGCCCTCTTGTGTCCCTCTGCTGTGGCTGTGGTCTCCATTCTCAGTACCTGTTTGATG 41 T N S L G R A D K E M R L T D G E D N C 121 ACCAATTCTCTTGGGAGAGCAGATAAAGAGATGAGGCTAACGGATGGTGAAGACAATTGC 61 S G R V E V K V Q E E W G T V C N N G W 181 TCCGGGAGAGTGGAAGTGAAAGTCCAGGAGGAGTGGGGAACGGTGTGTAATAATGGCTGG 81 G M D E V S V I C R Q L G C P T A I K A 241 GGCATGGATGAAGTCTCTGTGATTTGCAGGCAGCTGGGATGTCCCACTGCTATCAAAGCC 101 A G W A N S R A G S G R I W M D H V S C 301 GCTGGATGGGCCAATTCCAGGGCAGGCTCTGGACGAATCTGGATGGATCATGTTTCTTGT 121 R G N E S A L W D C K H D G W G K H N C 361 CGAGGGAATGAATCTGCTCTCTGGGACTGCAAACATGATGGATGGGGAAAGCACAACCTGC 141 S H Q Q D A G V T C S D G S S L E M R L 421 AGTCATCAACAGGATGCTGGAGTAACCTGTTTCAGATGGATCCAGTTTGGAGATGAGGTTG 161 M N G G N Q C S G R I E V K F Q G Q W G 481 ATGAACGGCGGAAACCAGTGTTCTGGCAGAATAGAAGTCAAGTTCAGGGACAGTGGGGA 181 T V C D D N F N I D H A S V V C K Q L E 541 ACAGTGTGTGATGACAACTTCAACATAGATCATGCTTCTGTGGTTTGTAAACAGCTCGAA 201 C G S A V S F S G S A N F G E G S G P I 601 TGTGGAAGTGCTGTTCAGTTTCTCTGGTTTCAGCTAATTTTGGAGAAGGTTCTGGGCCAATC 221 W F D D L V C S G N E S A L W N C K H E 661 TGGTTTGATGATCTTGTGTGCAGTGGAAATGAGTCAGCTCTCTGGAACTGCAAGCATGAA 241 G W G K H N C D H A E D V G V I C L D G 721 GGATGGGGAAAGCATAACTGTGATCACGCTGAGGATGTTGGAGTGATTGCTTGGATGGA 261 A D L S L R L V D G V T E C S G R L E V 781 GCAGATCTGAGCCTGAGACTGGTAGATGGAGTCACTGAATGTTTCAGGAAGATTAGAAGTA 281 K F Q G E W G T V C D D G W D S N D A A 841 AAATTCCAAGGGGAATGGGGGACAGTGTGTGATGATGGCTGGGATAGTAATGATGCTGCT 301 V V C K Q L G C P T A V T A I G R V N A 901 GTGGTATGTAAACAACTGGGATGCCCAACTGCTGTCACCGCCATTGGTCGAGTTAACGCC 321 S E G S G H I W L D N L S C Q G D E S A	SEQ ID NO: 45 and 46

961 AGTGAGGGAAGTGGACACATTTGGCTTGACAATCTTTCCTGCCAAGGAGACGAATCTGCT
 341 L W Q C R H H E W G K H Y C N H N E D A
 1021 CTCTGGCAGTGTAGACACCATGAATGGGGAAAGCATTATTGCAATCATAATGAAGATGCT
 361 G V T C S D G S D L E L R L V G G G S R
 1081 GGTGTGACATGTTCTGATGGATCAGACCTGGAGCTGAGACTTGTCTGGTGGAGGCAGCCGC
 381 C A G T V E V E I Q K L L G K V C D R G
 1141 TGTGCTGGGACAGTGGAGGTTGAAATTCAGAACTGCTAGGGAAAGTATGTGATAGAGGC
 401 W G L K E A D V V C K Q L G C G S A L K
 1201 TGGGGACTGAAAGAAGCCGATGTGGTTTGCAAGCAGTTGGGATGTGGATCTGCTCTCAA
 421 T S Y Q R Y S K V K A T N T W L F L S R
 1261 ACGTCCTATCAGCGTTATTCCAAAGTTAAGGCAACAAACACATGGCTGTTTTTAAGCCGC
 441 C S G N E T S L W D C K N W Q W G G L S
 1321 TGTAGTGGCAATGAAACTTCCCTTTGGGACTGCAAGAACTGGCAGTGGGGTGGACTGAGC
 461 C D H Y E E A K V T C S A H R E P R L V
 1381 TGTGATCACTATGAAGAAGCTAAAGTTACCTGCTCAGCCACAGGGAACCCAGACTAGTT
 481 G G D I P C S G R V E V K H G D T W G T
 1441 GGAGGAGATATTCCTGCTCTGGTCGTGTTGAAGTGAAACATGGTGACACATGGGGCACC
 501 V C D S D F S L E A A S V L C R E L Q C
 1501 GTCTGTGATTCGACTTCTCTTTGGAAGCTGCCAGTGTGCTGTGCAGAGAGTTACAGTGT
 521 G T V I S I L G G A H F G E G N G Q I W
 1561 GGCACAGTCATCTCCATCCTAGGGGGAGCTCACTTTGGAGAAGGAAATGGACAGATCTGG
 541 A E E F Q C E G Q E S H L S L C S V A S
 1621 GCTGAAGAATTCCAGTGTGAGGGGCAGGAGTCCCATCTTTCAGTCTGTTTACAGTAGCCTCT
 561 R P D G T C S H S R D V G V V C S R Y T
 1681 CGCCCAGATGGGACCTGTAGCCACAGCAGGGATGTTGGAGTCGTCTGCTCAAGATACACG
 581 E I R L V N G Q S P C E G R V E L K I L
 1741 GAAATCCGCTTGGTGAATGGCCAGTCCCCGTGTGAAGGAAGAGTGGAGCTCAAGATACTT
 601 G N W G S L C N S H W D I E D A H V F C
 1801 GGGAAGTGGGGATCCCTCTGCAACTCTCACTGGGACATAGAAGATGCCCATGTTTTCTGT
 621 Q Q L K C G V A L S I P G G A H F G K G
 1861 CAGCAGCTCAAATGTGGAGTTGCCCTTTCTATTCCGGGAGGAGCACATTTTGGGAAAGGA
 641 S G Q I W R H M F H C T G T E Q H M G D
 1921 AGTGGTCAGATCTGGAGGCACATGTTTCACTGCACTGGGACTGAGCAGCACATGGGAGAT
 661 C P V T A L G A T L C S A G Q V A S V I
 1981 TGCCCTGTAAGTCTGCTGCGGCGACGCTGTGTTCTGCTGGGCAAGTGGCCTCTGTAATC
 681 C S G N Q S Q T L S P C N S T S L D P T
 2041 TGCTCAGGAAATCAGAGCCAGACGCTATCCCCATGCAATTCAACATCTCTGGACCCAACA
 701 R S T T S E E S A V A C I A S G Q L R L
 2101 AGATCTACCACTTCGGAAGAAAGTGTGTTGCTTGTATTGCGAGTGGGCAACTTCGCTG
 721 V N G G G R C A G R I E V Y H E G S W G
 2161 GTAAATGGAGGCGGTCGCTGTGCTGGGAGAATAGAGGTCTACCATGAGGGCTCCTGGGGC
 741 T I C D D S W D L S D A H V V C R Q L G
 2221 ACCATCTGTGATGACAGCTGGGACCTGAGTGATGCCCATGTGGTGTGCAGACAGCTGGGC
 761 C G V A I N A T G S A H F G E G T G P I
 2281 TGTGGAGTGGCCATTAATGCCACTGGCTCTGCTCATTTTGGGGAAGGAACAGGGCCCATC
 781 W L D E V N C N G K E S H I W Q C R S H
 2341 TGGCTGGACGAGGTGAACTGTAATGGAAAGGAATCTCATATCTGGCAATGCCGCTCACAC
 801 G W G Q H N C R H K E D A G V I C S E F
 2401 GGCTGGGGGCAACACAAGTGCAGACATAAGGAGGATGCAGGAGTTATCTGCTCAGAGTTC
 821 M S L R L I D E T S R D I C A G R L E V
 2461 ATGTCTCTCAGACTGATTGATGAAACCAGCAGAGACATCTGTGCAGGGCGTCTTGAAGTT
 841 F Y N G A W G S V G K S N M S A T T V E
 2521 TTTTACAATGGAGCTTGGGGCAGCGTTGGCAAGAGTAATATGTCTGCAACCACTGTGGAG
 861 V V C R Q L G C A D K G S I N P A S S D
 2581 GTGGTATGCAGGCAACTGGGTTGTGCAGACAAGGGGAGCATCAACCCTGCATCTTCAGAC

881 K P M S R H M W V D N V Q C P K G P D T 2641 AAGCCCATGTCCAGGCACATGTGGGTGGACAATGTCCAGTGTCCAAAAGGACCTGACACC 901 L W Q C P S S P W K Q R V A S S S E E T 2701 TTATGGCAGTGGCCATCTTCTCCATGGAAACAGAGAGTGGCCAGTTCTTCAGAGGAGACC 921 W I T C A N K I R L Q E G T S N C S G R 2761 TGGATCACATGTGCCAACAAGATAAGACTTCAAGAAGGAACCTCTAATTGTTCTGGACGT 941 V E L W H G G S W G T V C D D S W D L E 2821 GTGGAGCTCTGGCACGGAGGTTCTGGGGGACAGTGTGCGATGACTCCTGGGACCTTGAA 961 D A Q V V C R Q L G C G P A L E A L K E 2881 GATGCACAAGTGGTGTGTGCGACAGCTGGGCTGTGGCCAGCATTAGAAGCACTAAAAGAG 981 A A F G Q G T G P I W L N D V K C K G N 2941 GCAGCATTTGGTCAGGGGACTGGGCCTATATGGCTCAATGACGTGAAGTGCAAAGGGAAT 1001 E S S L W D C P A R P W G H S D C G H K 3001 GAGTCTTCCTTGTGGGATTGTCCTGCTAGACCCTGGGGGCACAGTGACTGTGGCCACAAG 1021 E D A A V R C S E I A M A Q R S S N P R 3061 GAAGATGCTGCTGTGAGGTGCTCAGAAATTGCAATGGCCCAAAGATCATCAAATCCTAGA 1041 G H S S L V A L G I F G V I L L A F L I 3121 GGTCACTCATCCCTTGTTCATTGGGGATCTTTGGTGTCAATCTTCTGGCCTTTCTCATC 1061 A L L L W T Q R R R Q Q Q R L T V S L R 3181 GCTCTCTCTTGTGGACTCAAAGGCGAAGACAGCAACAGCGGCTTACAGTTTCCTTGAGA 1081 G E N S V H Q I Q Y R E M N S S L K A D 3241 GGAGAGAATTCTGTCCACCAAATTCAATACGGGAAATGAATTCTTCCTGAAAGCAGAT 1101 D L D V L T S S E D H F E V H 3301 GATCTGGACGTGCTGACTTCCTCAGAAGACCATTTTGTAGGTACAC	
1 MSKLRMVPHG NSGSADFRR C FALLCPSAVA VVSILSTCLM TNSLGRADKE MRLTDGEDNC 61 SGRVEVKVQE EWGTVCNNGW GMDEVSVICR QLGCPTAIKA AGWANSRAGS GRIWMDHVSC 121 RGNESALWDC KHDGWGKHNC SHQQDAGVTC SDGSSLEMRL MNGGNQCSGR IEVKFQGQWG 181 TVCDDNFNID HASVVCKQLE CGSAVSFSGS ANFGEGSGPI WFDDLVCNGN ESALWNCKHE 241 GWGKHNCDDHA EDVGVICLDG ADLSLRLVDG VTECSGRLEV KFQGEWGTVC DDGWDSNDAA 301 VVCKQLGCPT AVTAIGRVNA SEGSGHIWLD NLSCQGDSEA LWQCRHHEWG KHYCNHNEDA 361 GVTCSGSDL ELRLVGGGSR CAGTVEVEIQ KLLGKVCDRG WGLKEADVVC KQLGCGSALK 421 TSYQRYSKVK ATNTWLFLSR CSGNETSLWD CKNWQWGLS CDHYEEAKVT CSAHREPRLV 481 GGDIPCSGRV EVKHGDTWGT VCDSDFSLEA ASVLCRELQC GTVISILGGA HFGEGNGQIW 541 AEEFQCEGQE SHLSLCSVAS RPDGTCSSHR DVGVCSSRYT EIRLVNGQSP CEGRVELKIL 601 GNWGSCLNSH WDIEDAHVFC QQLKCGVALS IPGGAHFGKG SGQIWRHMFH CTGTEQHMGD 661 CPVTALGATL CSAGQVASVI CSGNQSQTLS PCNSTSLDPT RSTTSEESAV ACIASGQLRL 721 VNGGGRGAGR IEVYHEGSWG TICDDSWDLS DAHVVCRLQG CGVAINATGS AHFGEGTGPI 781 WLDEVNCNGK ESHIWQCRSH GWGQHNCRHK EDAGVICSEF MSLRLIDETS RDICAGRLEV 841 FYNGAWGSVG KSNMSATTVE VVCRQLGCAD KGSINPASSD KPMSRHMWVD NVQCPKGPDT 901 LWQCPSSPWK QRVASSSEET WITCANKIRL QEGTSNCSGR VELWHGGSWG TVCDDSWDLE 961 DAQVVCRQLG CGPALEALKE AAFGQGTGPI WLNDVKCKGN ESSLWDPCPAR PWGHSDCGHK 1021 EDAAVRCSEI AMAQRSSNPR GHSSLVALGI FGVILLAFLL ALLLWTQRRR QQQLRTVSLR 1081 GENSVHQIQY REMNSSLKAD DLDVLTSSD HFEVH	SEQ ID NO:46
1 M S K L R M V P H G N S G S A D F R R C 1 ATGAGCAAACCTCAGAATGGTCCCACATGGAACTCTGGATCTGCTGACTTTAGAAGATGT 21 F A L L C P S A V A V V S I L S T C L M 61 TTTGCCCTCTTGTGTCCCTCTGCTGTGGCTGTGGTCTCCATTCTCAGTACCTGTTTGATG 41 T N S L G R A D K E M R L T D G E D N C 121 ACCAATTCTCTTGGGAGAGCAGATAAAGAGATGAGGCTAACGGATGGTGAAGACAATTGC 61 S G R V E V K V Q E E W G T V C N N G W 181 TCCGGGAGAGTGGAAGTGAAAGTCCAGGAGGAGTGGGGAACGGTGTGTAATAATGGCTGG 81 G M D E V S V I C R Q L G C P T A I K A 241 GGCATGGATGAAGTCTCTGTGATTTGCAGGCAGCTGGGATGTCCCACTGCTATCAAAGCC 101 A G W A N S R A G S G R I W M D H V S C 301 GCTGGATGGGCCAATTCCAGGGCAGGCTCTGGACGAATCTGGATGGATCATGTTTCTTGT 121 R G N E S A L W D C K H D G W G K H N C 361 CGAGGGAATGAATCTGCTCTCTGGGACTGCAAACATGATGGATGGGGAAGCACAACTGC 141 S H Q Q D A G V T C S D G S S L E M R L	SEQ ID NO:47

421 AGTCATCAACAGGATGCTGGAGTAACCTGTTTCAGATGGATCCAGTTTGGAGATGAGGTTG
 161 M N G G N Q C S G R I E V K F Q G Q W G
 481 ATGAACGGCGGAAACCAGTGTCTGGCAGAATAGAAGTCAAGTTCAGGGACAGTGGGGA
 181 T V C D D N F N I D H A S V V C K Q L E
 541 ACAGTGTGTGATGACAACTTCAACATAGATCATGCTTCTGTGGTTTGTAAACAGCTCGAA
 201 C G S A V S F S G S A N F G E G S G P I
 601 TGTGGAAGTGCTGTCTAGTTTCTCTGGTTTCAGCTAATTTTGGAGAAGGTTCTGGGCCAATC
 221 W F D D L V C S G N E S A L W N C K H E
 661 TGGTTTGATGATCTTGTGTGCAGTGGAAATGAGTCAGCTCTCTGGAAGTCAAGCATGAA
 241 G W G K H N C D H A E D V G V I C L D G
 721 GGATGGGGAAAGCATAACTGTGATCAGCTGAGGATGTTGGAGTGATTTGCTTGGATGGA
 261 A D L S L R L V D G V T E C S G R L E V
 781 GCAGATCTGAGCCTGAGACTGGTAGATGGAGTCACTGAATGTTTCAGGAAGATTAGAAGTA
 281 K F Q G E W G T V C D D G W D S N D A A
 841 AAATTCCAAGGGGAATGGGGGACAGTGTGTGATGATGGCTGGGATAGTAATGATGCTGCT
 301 V V C K Q L G C P T A V T A I G R V N A
 901 GTGGTATGTAAACAACTGGGATGCCCAACTGCTGTCACCGCCATTGGTCGAGTTAACGCC
 321 S E G S G H I W L D N L S C Q G D E S A
 961 AGTGAGGGAAGTGGACACATTTGGCTTGACAATCTTTCCTGCCAAGGAGACGAATCTGCT
 341 L W Q C R H H E W G K H Y C N H N E D A
 1021 CTCTGGCAGTGTAGACACCATGAATGGGGAAAGCATTATTGCAATCATAATGAAGATGCT
 361 G V T C S D G S D L E L R L V G G G S R
 1081 GGTGTGACATGTTCTGATGGATCAGACCTGGAGCTGAGACTTGTCTGGTGGAGGCAGCCGC
 381 C A G T V E V E I Q K L L G K V C D R G
 1141 TGTGCTGGGACAGTGGAGGTTGAAATTCAGAACTGCTAGGGAAAGTATGTGATAGAGGC
 401 W G L K E A D V V C K Q L G C G S A L K
 1201 TGGGGACTGAAAGAAGCCGATGTGGTTTGCAAGCAGTTGGGATGTGGATCTGCTCTCAA
 421 T S Y Q R Y S K V K A T N T W L F L S R
 1261 ACGTCCTATCAGCGTTATTCCAAAGTTAAGGCAACAAACACATGGCTGTTTTTAAGCCGC
 441 C S G N E T S L W D C K N W Q W G G L S
 1321 TGTAAGTGGCAATGAAACTTCCCTTTGGGACTGCAAGAACTGGCAGTGGGGTGGACTGAGC
 461 C D H Y E E A K V T C S A H R E P R L V
 1381 TGTGATCACTATGAAGAAGCTAAAGTTACCTGCTCAGCCCACAGGGAACCCAGACTAGTT
 481 G G D I P C S G R V E V K H G D T W G T
 1441 GGAGGAGATATTCCTGCTCTGGTCGTGTTGAAGTGAACATGGTGACACATGGGGCACC
 501 V C D S D F S L E A A S V L C R E L Q C
 1501 GTCTGTGATTCCGACTTCTCTTTGGAAGCTGCCAGTGTGCTGTGCAGAGAGTTACAGTGT
 521 G T V I S I L G G A H F G E G N G Q I W
 1561 GGCACAGTCATCTCCATCCTAGGGGGAGCTCACTTTGGAGAAGGAAATGGACAGATCTGG
 541 A E E F Q C E G Q E S H L S L C S V A S
 1621 GCTGAAGAATTCCAGTGTGAGGGGCAGGAGTCCCATCTTTCAGTCTGTTTCAGTAGCCTCT
 561 R P D G T C S H S R D V G V V C S R Y T
 1681 CGCCCAGATGGGACCTGTAGCCACAGCAGGGATGTTGGAGTCGTCTGCTCAAGATACACG
 581 E I R L V N G Q S P C E G R V E L K I L
 1741 GAAATCCGCTTGGTGAATGGCCAGTCCCGTGTGAAGGAAGAGTGGAGCTCAAGATACTT
 601 G N W G S L C N S H W D I E D A H V F C
 1801 GGGAAGTGGGGATCCCTCTGCAACTCTCACTGGGACATAGAAGATGCCCATGTTTTCTGT
 621 Q Q L K C G V A L S I P G G A H F G K G
 1861 CAGCAGCTCAAATGTGGAGTTGCCCTTTCTATTCCGGGAGGAGCACATTTTGGGAAAGGA
 641 S G Q I W R H M F H C T G T E Q H M G D
 1921 AGTGGTCAGATCTGGAGGCACATGTTTCACTGCACTGGGACTGAGCAGCACATGGGAGAT
 661 C P V T A L G A T L C S A G Q V A S V I
 1981 TGCCCTGTAAGTCTCTGGGCGGACGCTGTGTTCTGCTGGGCAAGTGGCCTCTGTAATC
 681 C S G N Q S Q T L S P C N S T S L D P T
 2041 TGCTCAGGAAATCAGAGCCAGACGCTATCCCCATGCAATTCAACATCTCTGGACCCAACA

701 R S T T S E E S A V A C I A S G Q L R L	
2101 AGATCTACCACTTCGGAAGAAAGTGCTGTTGCTTGTATTGCGAGTGGGCAACTTCGCCTG	
721 V N G G G R C A G R I E V Y H E G S W G	
2161 GTAAATGGAGGCGGTCTGCTGTGCTGGGAGAATAGAGGTCTACCATGAGGGCTCCTGGGGC	
741 T I C D D S W D L S D A H V V C R Q L G	
2221 ACCATCTGTGATGACAGCTGGGACCTGAGTGATGCCCATGTGGTGTGCAGACAGCTGGGC	
761 C G V A I N A T G S A H F G E G T G P I	
2281 TGTGGAGTGGCCATTAATGCCACTGGCTCTGCTCATTCTTTGGGGAAGGAACAGGGCCCATC	
781 W L D E V N C N G K E S H I W Q C R S H	
2341 TGGCTGGACGAGGTGAACTGTAATGGAAAGGAATCTCATATCTGGCAATGCCGCTCACAC	
801 G W G Q H N C R H K E D A G V I C S E F	
2401 GGCTGGGGGCAACACAAGTGCAGACATAAGGAGGATGCAGGAGTTATCTGCTCAGAGTTC	
821 M S L R L I D E T S R D I C A G R L E V	
2461 ATGTCTCTCAGACTGATTGATGAAACCAGCAGAGACATCTGTGCAGGGCGTCTTGAAGTT	
841 F Y N G A W G S V G K S N M S A T T V E	
2521 TTTTACAATGGAGCTTGGGGCAGCGTTGGCAAGAGTAATATGTCTGCAACCACTGTGGAG	
861 V V C R Q L G C A D K G S I N P A S S D	
2581 GTGGTATGCAGGCAACTGGGTTGTGCAGACAAGGGGAGCATCAACCCTGCATCTTCAGAC	
881 K P M S R H M W V D N V Q C P K G P D T	
2641 AAGCCCATGTCCAGGCACATGTGGGTGGACAATGTCCAGTGTCCAAAAGGACCTGACACC	
901 L W Q C P S S P W K Q R V A S S S E E T	
2701 TTATGGCAGTGCCCATCTTCTCCATGGAAACAGAGAGTGGCCAGTTCTTCAGAGGAGACC	
921 W I T C A N K I R L Q E G T S N C S G R	
2761 TGGATCACATGTGCCAACAAGATAAGACTTCAAGAAGGAACCTCTAATTGTTCTGGACGT	
941 V E L W H G G S W G T V C D D S W D L E	
2821 GTGGAGCTCTGGCACGGAGGTTCTGGGGGACAGTGTGCGATGACTCCTGGGACCTTGAA	
961 D A Q V V C R Q L G C G P A L E A L K E	
2881 GATGCACAAGTGGTGTGTCGACAGCTGGGCTGTGGCCAGCATTAGAAGCACTAAAAGAG	
981 A A F G Q G T G P I W L N D V K C K G N	
2941 GCAGCATTGTTGGTCAGGGGACTGGGCCTATATGGCTCAATGACGTGAAGTGCAAAGGGAAT	
1001 E S S L W D C P A R P W G H S D C G H K	
3001 GAGTCTTCCTTGTGGGATTGTCCTGCTAGACCCTGGGGGCACAGTGAAGTGTGGCCACAAG	
1021 E D A A V R C S E I A M A Q R S S N P R	
3061 GAAGATGCTGCTGTGAGGTGCTCAGAAATTGCAATGGCCCAAAGATCATCAAATCCTAGA	
1041 G H S S L V A L G I F G V I L L A F L I	
3121 GGTCACTCATCCCTTGTTCATTGGGGATCTTTGGTGTGATTCTTCTGGCCTTTCTCATC	
1061 A L L L W T Q R R R Q Q Q R L T V S L R	
3181 GCTCTCCTTGTGGACTCAAAGGCGAAGACAGCAACAGCGGCTTACAGTTTCTTGAGA	
1081 G E N S V H Q I Q Y R E M N S S L K A D	
3241 GGAGAGAATTCTGTCCACCAAATTCAATACCGGGAAATGAATTCTCCCTGAAAGCAGAT	
1101 D L D V L T S S E Y P N E S D D F N D A	
3301 GATCTGGACGTGCTGACTTCCTCAGAATATCCCAATGAGTCAGATGATTTTAATGATGCT	
1121 G L I S V S K S L P I S G	
3361 GGGCTAATTTCTGTGTCTAAATCTCTTCTTCTGGA	
1 MSKLRMVPHG NSGSADFRR FALLCPSAVA VVSILSTCLM TNSLGRADKE MRLTDGEDNC	SEQ ID NO:48
61 SGRVEVKVQE EWGTVCNNGW GMDEVSVICR QLGCPTAIKA AGWANSRAGS GRIWMDHVSC	
121 RGNESALWDC KHDGWGKHNC SHQQDAGVTC SDGSSLEMRL MNGGNQCSGR IEVKFQGGWG	
181 TVCDDNFNID HASVVCCKLE CGSAVSFSGS ANFGEGSGPI WFDDLVCSEGN ESALWNCKHE	
241 GWGKHNCDDHA EDVGVICLDG ADLSLRLVDG VTECSGRLEV KFQGEWGTVC DDGWDSNDAA	
301 VVCKQLGCPT AVTAIGRVNA SEGSGHIWLD NLSCQGDSEA LWQCRHHEWG KHYCNHNEDA	
361 GVTCSGSDSL ELRLVGGGSR CAGTVEVEIQ KLLGKVCDRG WGLKEADVVC KQLGCGSALK	
421 TSYQRYSKVK ATNTWLFSLR CSGNETSLWD CKNWQWGGLS CDHYEEAKVT CSAHREPLV	
481 GGDIPCSGRV EVKHGDTWGT VCDSDFSLEA ASVLCRELQC GTVISILGGA HFGEGNGQIW	
541 AEEFQCEGQE SHLSLCSVAS RPDGTCSSHR DVGVCSSRYT EIRLVNGQSP CEGRVELKIL	
601 GNWGSCLNSH WDIEDAHVFC QQLKCGVALS IPGGAHFGKG SGQIWRHMFH CTGTEQHMGD	
661 CPVTALGATL CSAGQVASVI CSGNQSQTLN PCNSTSLDPT RSTTSEESAV ACIASGQLRL	
721 VNGGGRCAGR IEVYHEGSGW TICDDSWDLN DAHVVCRLG CGVAINATGS AHFGEGTGPI	
781 WLDEVNCGNK ESHIWQCRSH GWGQHNCRHK EDAGVICSEF MSLRLIDETS RDICAGRLEV	
841 FYNGAWGSVG KSNMSATTVE VVCRQLGCAD KGSINPASSD KPMSRHMWVD NVQCPKGPDT	

901	LWQCPSSPWK	QVASSSEET	WITCANKIRL	QEGTSNCSGR	VELWHGGSWG	TVCDDSWDLE
961	DAQVVCRLG	CGPALEALKE	AAFGQGTGPI	WLNDVKCKGN	ESSLWDCPAR	PWGHSDCGHK
1021	EDAAVRCSEI	AMAQRSSNPR	GHSSLVALGI	FGVILLAFLI	ALLLWTQRRR	QQQRLTVSLR
1081	GENSVHQIQY	REMNSSLKAD	DLDLVTSSEY	PNESDDFNDA	GLISVSKSLP	ISG

Example 11. Various cell lines are rendered permissive to North American PRRSV infection following transient transfection with pCMV-susCD163v1

5 Porcine Kidney (PK032495), Norden Labs Swine Testicular (NLST-1), Norden Labs Dog Kidney (NLDK-1) were obtained from Pfizer Inc. and were grown at 37° C and 5% CO₂ in growth media consisting of Dulbecco's Modified Eagle Medium (DMEM, Invitrogen catalog number 11965) supplemented with 5% fetal bovine serum (FBS), 1mM sodium pyruvate, 2mM L-glutamine and antibiotics. Cell lines Baby Hamster

10 Kidney (BHK21), Norden Labs Feline Kidney (NLFK-1), and Rabbit Lung (RL) were obtained from Pfizer Inc. and were grown at 37° C and 5% CO₂ in growth media consisting of Dulbecco's Modified Eagle Medium (DMEM, Invitrogen catalog number 11965) supplemented with 10% fetal bovine serum (FBS), 1mM sodium pyruvate, 2mM L-glutamine and antibiotics. Vero cells were obtained from Pfizer

15 Inc. and were grown at 37° C and 5% CO₂ in growth media consisting of Minimum Essential Medium Alpha (MEM, Pfizer Inc. formulation) supplemented with 10% fetal bovine serum (FBS), 2mM L-glutamine and Gentamicin at 20 micrograms per mL. Cell culture wells (35 mm) containing approximately 1x10⁶ cells were transfected with 2 micrograms per well of plasmid pCMV-susCD163v1, in DMEM

20 without FBS or antibiotics, using Lipofectamine 2000 (Invitrogen catalog number 11668-027) according to the manufacturer's instructions. Cell line RL was transfected with 1.0 micrograms per well of plasmid pCMV-susCD163v1. A member of the PAM cell cDNA library without an insert, designated pPAMB (essentially and empty pSport plasmid vector), was used as a negative control plasmid. At 24 hours

25 post transfection wells were aspirated and washed twice with DMEM/5% FBS followed by infection with North American PRRSV isolate P129. Virus was allowed to adsorb in 0.5 ml growth media for a minimum of two hours, after which additional media was added to a final volume of 2.0ml and incubated overnight. The virus was then removed, wells washed twice with growth media, and fresh growth media added

30 (2.0 ml per well). A time zero sample of culture fluid was immediately taken in order to determine the background level of infectious virus from the inoculum. At a minimum of 48 hours post infection cultures were screened for permissivity by removing culture fluids in order to assay viable virus, and permissive cells in the

monolayer were detected by fluorescent antibody assay (FA). The FA was completed by fixing the monolayer with 80% acetone and stained with FITC-conjugated monoclonal antibody SDOW17 (Rural Technologies Inc), which is specific for the PRRSV nucleocapsid protein. Viable virus was titrated by inoculating dilutions of culture fluids onto MARC-145 cells. Table 5 shows the results of virus infection by FA and the presence of progeny virus for each cell line tested.

Failure to detect progeny virus from some cell lines may be the result of low virus titer in the cell culture fluids, below the assay's limit of detection. Permissivity of Vero cells to PRRSV infection was augmented by the expression of susCD163v1. Compared to the time zero measurement of background virus, there was nearly a two-log increase in virus titers in Vero cells transfected with pCMV-susCD163v1, whereas there was less than a one-log in titer increase in cells transfected with negative control plasmid pPAMB. All cell lines except NLDK-1 were positive by FA for permissivity to North American PRRSV isolate P129 infection after transfection with pCMV-susCD163v1.

Table 5

Screening of various cell lines for permissivity to NA PRRSV isolate P129 following transient transfection with pCMV-susCD163v1 or pPAMB				
Transfected cell line	Fluorescent Antibody assay		Progeny virus produced	
	pCMV-susCD163v1	pPAMB	pCMV-susCD163v1	pPAMB
BHK21	+++	-	+++	-
PK032495	+	-	+	-
NKFK-1	+	-	+	-
NLST-1	+	-	-	-
NLDK-1	-	-	NT	NT
RL	+	-	-	-
Vero	++	+	++	+

+++ = Highly positive

++ = Moderately positive

20 + = Slightly positive

- = Not detectible

NT = Not tested

25 **Example 12. BHK21 cells are rendered permissive to European PRRSV infection following transient transfection with pCMV-susCD163v1**

Cell line Baby Hamster Kidney (BHK21) was obtained from Pfizer Inc. and grown at 37° C and 5% CO₂ in growth media consisting of Dulbecco's Modified Eagle Medium

(DMEM, Invitrogen catalog number 11965) supplemented with 10% fetal bovine serum (FBS), 1mM sodium pyruvate, 2mM L-glutamine and antibiotics. Cell culture wells (35 mm) containing approximately 1×10^6 cells were transfected with 2 micrograms per well of plasmid pCMV-susCD163v1, in DMEM without FBS or antibiotics, using Lipofectamine 2000 (Invitrogen catalog number 11668-027) according to the manufacturer's instructions. At 24 hours post transfection wells were aspirated and washed twice with DMEM/5% FBS followed by infection with European PRRSV isolate 96V198. Virus was allowed to adsorb for a minimum of 2 hours. The virus was then removed, wells washed twice with growth media, and fresh growth media added (2.0 ml per well). A time zero sample of culture fluid was immediately taken in order to determine the background level of infectious virus from the inoculum. At a minimum of 48 hours post infection cultures were screened for permissivity by removing culture fluids in order to assay viable virus, and permissive cells in the monolayer were detected by fluorescent antibody assay (FA). The FA was completed by fixing the monolayer with 80% acetone and stained with FITC-conjugated monoclonal antibody SDOW17 (Rural Technologies Inc), which is specific for the PRRSV nucleocapsid protein. Viable virus was titrated by inoculating dilutions of culture fluids onto MARC-145 cells. As a result of the transient transfection of BHK21 with pCMV-susCD163v1, cells were rendered permissive to European PRRSV isolate 96V198 infection and yielded progeny virus.

Example 13: CD163 genes from multiple animal species render BHK21 cells permissive to PRRS virus infection

BHK21 cells grown in DMEM (Invitrogen catalog number 11965) supplemented with 10% fetal bovine serum, 1mM sodium pyruvate, and antibiotics, were used in transient transfection experiments. Before transfection cells were washed once with OptiMEM (Invitrogen) without serum or other additives. Lipofectamine 2000 (Invitrogen) was used in all transfection experiments according to the protocol provided by the manufacturer. The transfection mixture consisted of 10 microliters of Lipofectamine 2000 and 2-3 micrograms of DNA per 35 mm well. After overnight incubation, transfection medium was removed and cells were infected with PRRSV isolate P129. Infection was allowed to progress for 24-48 hours, when cells were fixed with 80% acetone and stained with monoclonal antibody SDOW17 conjugated

with FITC (Rural Technology Inc., Brookings, SD). Staining of the nucleocapsid protein was visualized under a fluorescence microscope.

Table 6. Transient transfection of BHK21 cells with various CD163 genes renders them permissive to PRRS virus infection

5

Table 6

Plasmid backbone	CD163 gene	PRRSV infection (FA)
pCMV-Script	Swine CD163v1	+++
pRSV-Script	Swine CD163v1	+++
pcDNA3.1D	Swine CD163v2	++
pcDNA3.1D	Human CD163v2	++
pcDNA3.1D	Mouse CD163v3	+
pcDNA3.1D	African green monkey (MARC-145 cell) CD163v2	+++
pcDNA3.1D	Vero cells CD163v7	+++
pcDNA3.1D	DH82 cell CD163v2	+++

+++ = Highly positive

++ = Moderately positive

+ = Slightly positive

10 **Example 14. Generation of PRRSV-permissive BHK21 stable cell lines using pCMV-susCD163v1**

BHK-21 cells were grown in Dulbecco's Modified Eagle Media (DMEM) supplemented with 10% fetal bovine serum, 1mM sodium pyruvate, and antibiotics. For transfection, cells were seeded at approximately 90% confluency in 6 well plates and incubated over night at 37°C in 5% CO₂. Cells were transfected with pCMV-susCD163v1 DNA using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. One day after transfection the cells were trypsinized and re-seeded in 96 well plates in a dilution series. To select for stable transfectants, the media was supplemented with 1 mg/ml Geneticin (G418 sulfate, Invitrogen catalog number 10131-027) from this point forward. Medium was changed every 3-5 days. Plates were cultured until those wells with colonies derived from single cells reached confluency, at which point the plates were trypsinized and seeded into duplicate 96 well plates. One of the duplicate 96 well plates was infected with PRRSV isolate P129 and clones permissive to infection were identified by staining with FITC conjugated monoclonal antibody SDOW17. Positive clones were then expanded from the second duplicate plate. To ensure homogeneity the positive cultures were single-cell cloned by limiting dilution. At each cloning the subclones that displayed robust

25

growth and high PRRSV permissivity were chosen for expansion. Three clones designated BHK/CMV/v1 #3, BHK/CMV/v1 #5, and BHK/CMV/v1 #12 (Figure 6) were selected. These cell lines have maintained the permissive phenotype through 20 passages.

5 **Example 15. Generation of PRRSV-permissive BHK21 stable cell lines using pRSV-susCD163v1**

BHK-21 cells were cultured as described in Example 14. BHK-21 cells were transfected with pRSVsusCD163v1 using Lipofectamine 2000 as described in Example 14. Cloning of transfected cells and screening for permissive clones was
10 performed essentially as described in Example 14. From the original cloning 3 single cell clones were identified as permissive and were subsequently recloned two more times to ensure homogeneity and to attempt to isolate subclones of higher permissivity (see Figure 7). The resulting cell lines were named BHK/RSV/v1, #2, #3, and #4. All of these clones have maintained the permissive phenotype through the highest passage
15 tested (passage 11 for clone #2, passage 7 for clone #3, and passage 5 for clone #4).

Example 16. Generation of PRRSV-permissive feline kidney stable cell lines using pCMV-susCD163v1

Parental Norden Labs Feline Kidney (NLFK) cells were grown at 37 degrees C and 5% CO₂ in Dulbecco's Modified Eagle Medium (Invitrogen catalog number
20 11965-092) supplemented with 10% fetal bovine serum, 1 mM sodium pyruvate, and antibiotics. Several 35 mm wells containing approximately 2x10⁶ cells each were transfected with 4 micrograms per well of pCMV-susCD163v1, in OptiMEM, using Lipofectamine 2000 (Invitrogen catalog number 11668-027) according to the manufacturer's instructions. After overnight incubation, cells were removed from the
25 substrate using Accutase (Innovative Cell Technologies, catalog number AT104) diluted in medium, and seeded into three 96-well plates at three densities (approximately 2 x 10², 2 x 10³, and 2 x 10⁴ cells per well). The cells were allowed to settle overnight at 37 degrees C before beginning selection of stable transformants. The next morning medium was replaced with 100 microliters / well fresh medium
30 containing 500 micrograms / ml Geneticin (G418 sulfate, Invitrogen catalog number 10131-027) to select for cells expressing the neomycin resistance gene. Medium was changed every 2 or 3 days to maintain Geneticin potency. After 19 days of selection, the 96-well plate with the lowest initial cell density (approximately 200 cells / well) yielded 70 empty wells and 26 wells with one or more colonies of G418-resistant cells

(calculated number of resistant cells / well is 0.3, using the Poisson distribution).

These 26 wells were split into duplicate wells and allowed to settle overnight. One set of wells was infected with PRRSV isolate P129, incubated for 24 hours, then fixed with 80% acetone and stained with FITC-conjugated monoclonal antibody SDOW17 (Rural Technologies Inc), which is specific for PRRSV nucleocapsid. Of the 26 clones, 8 contained some cells that were infected by PRRSV. One of these, designated "NLFK-CMV-susCD163v1-G4", was clearly more permissive than the others with nearly 100% of the cells staining positive for viral antigen.

By cell passage number 5, there was some evidence of phenotypic heterogeneity in the NLFK-CMV-susCD163v1-G4 cell line. Therefore, the cells were single-cell cloned by limiting dilution in G418-containing medium, starting with frozen stocks of NLFK-CMV-susCD163v1-G4 passage 4. Twelve such clones ("A" – "L") were expanded for study. Of these, clones NLFK-CMV-susCD163v1-G4F and NLFK-CMV-susCD163v1-G4L were notable for their ability to form discrete plaques (localized areas of CPE) when infected with PRRSV isolate P129 (see Figure 8).

Example 17. Generation of PRRSV-permissive feline kidney stable cell lines using pRSV-susCD163v1

Norden Labs Feline Kidney (NLFK) cells were grown at 37° C and 5% CO₂ in Minimal Essential Medium Alpha Medium (Invitrogen catalog number 12571-071) supplemented with 10% fetal bovine serum and antibiotics. NLFK cells were seeded in 6 well plates at approximately 90% confluency and allowed to attach overnight. The cells were then transfected with plasmid pRSV-susCD163v1 using Lipofectamine 2000 (Invitrogen) following the manufacturer's instructions. After 24 hours the cells were cloned as described in Example 14. Screening for PRRSV permissive cell clones was performed as described in Example 14. Four clones were selected from the screening and were single cell cloned by limiting dilution two more times. Four clones named FK/RSV/v1 #1, FK/RSV/v1 #2, FK/RSV/v1 #3, and FK/RSV/v1 #4 were selected. These cell lines have maintained the PRRSV permissive phenotype through at least 8 passages (see Figure 9).

Example 18. Generation of PRRSV-permissive porcine kidney stable cell lines using pCMV-susCD163v1

Parental Porcine Kidney (PK032495) cells were obtained from Pfizer Inc. and were grown at 37 degrees C and 5% CO₂ in growth media consisting of Dulbecco's

Modified Eagle Medium (DMEM, Invitrogen catalog number 11965) supplemented with 5% fetal bovine serum (FBS), 1mM sodium pyruvate, 2mM L-glutamine and antibiotics. Tissue culture wells (35 mm) containing approximately 1×10^6 cells each were transfected with 2 micrograms per well of plasmid pCMV-susCD163v1, in
5 DMEM without FBS or antibiotics, using Lipofectamine 2000 (Invitrogen catalog number 11668-027) according to the manufacturer's instructions. After overnight incubation, cells were washed with PBS and removed from the substrate using Accutase (Innovative Cells Technologies, catalog number AT104) and diluted in growth medium containing Geneticin (G418 sulfate, Invitrogen catalog number
10 10131-027) at 1.0 milligram per ml and seeded into 96-well plates at various densities to ensure recovery of single cell clones after Geneticin selection. Throughout Geneticin selection, media was changed approximately every 3 to 5 days. After selection, wells containing single cell clones were expanded into duplicate 96-well plates and allowed to incubate until 100% confluency was achieved. One set of wells
15 was screened for PRRSV-permissivity by infecting with PRRSV isolate P129 for a minimum of 48 hours. Eleven clones were found to be permissive for PRRSV. One of these, designated "PK-CMV-susCD163v1-A10", clearly retained the permissive phenotype after numerous passages (see Figure 10).

Example 19. Generation of PRRSV-permissive BHK21 stable cell lines using
20 **pCMVScript-susCD163v2**

Parental Baby Hamster Kidney (BHK21) cells were obtained from Pfizer Inc. and were grown at 37 degrees C and 5% CO₂ in growth media consisting of Dulbecco's Modified Eagle Medium (DMEM, Invitrogen catalog number 11965) supplemented with 10% fetal bovine serum (FBS), 1mM sodium pyruvate, 2mM L-
25 glutamine and antibiotics. Tissue culture wells (35 mm) containing approximately 1×10^6 cells each were transfected with 2 micrograms per well of pCMVScript-susCD163v2, in DMEM without FBS or antibiotics, using Lipofectamine 2000 (Invitrogen catalog number 11668-027) according to the manufacturer's instructions. After overnight incubation, cells were washed with PBS and removed from the
30 substrate using Accutase (Innovative Cells Technologies, catalog number AT104) and diluted in growth medium containing Geneticin (G418 sulfate, Invitrogen catalog number 10131-027) at 1.0 milligram per ml and seeded into 96-well plates at various densities to ensure recovery of single cell clones after Geneticin selection.

Throughout Geneticin selection, media was changed approximately every 3 to 5 days. After selection, wells containing single cell clones were expanded into duplicate 96-well plates and incubated until 100% confluency was achieved. One set of wells was screened for permissivity by infecting with PRRSV isolate P129 and incubating for a minimum of 48 hours. Three clones were found to be PRRSV-permissive, and one of these, designated "BHK-CMVScript-susCD163v2-A9", was chosen for further study (see Figure 11).

Example 20. Generation of PRRSV-permissive BHK-21 stable cell lines using pRSV-susCD163v2

BHK-21 cells were cultured as described in Example 14. BHK-21 cells were transfected with the ligated pRSV-susCD163v2 DNA construct described in Example 5 using Lipofectamine 2000 (Invitrogen) following manufacture's instructions. Subsequent cloning and selection of PRRSV permissive cell lines was performed as described in Example 14. Of 336 single cell clones screened, 129 were positive. Several of these cell clones have been passed up to 7 times and they have maintained the PRRSV permissive phenotype (see Figure 12). These cell lines have been named BHK/RSV/v2, followed by a numerical clone number.

Example 21. Generation of PRRSV-permissive porcine kidney stable cell lines using pCMVScript-susCD163v2

Parental Porcine Kidney (PK032495) cells were obtained from Pfizer Inc. and were grown at 37 degrees C and 5% CO₂ in growth media consisting of Dulbecco's Modified Eagle Medium (DMEM, Invitrogen catalog number 11965) supplemented with 5% fetal bovine serum (FBS), 1mM sodium pyruvate, 2mM L-glutamine and antibiotics. Tissue culture wells (35 mm) containing approximately 1x10⁶ cells each were transfected with 2 micrograms per well of pCMVScript-susCD163v2 in DMEM without FBS or antibiotics, using Lipofectamine 2000 (Invitrogen catalog number 11668-027) according to the manufacturer's instructions. After overnight incubation, cells were washed with PBS and removed from the substrate using Accutase (Innovative Cells Technologies, catalog number AT104) and diluted in growth medium containing Geneticin (G418 sulfate, Invitrogen catalog number 10131-027) at 1.0 milligram per ml and seeded into 96-well plates at various densities to ensure recovery of single cell clones after Geneticin selection. Throughout Geneticin selection, media was changed approximately every 3 to 5 days. After selection, wells

containing single cell clones were expanded into duplicate 96-well plates and incubated until 100% confluency was achieved. One set of wells was screened for permissivity by infecting with PRRSV isolate P129 and incubating for a minimum 48 hours. One clone designated "PK-CMVScript-susCD163v2-D1" showed the PRRSV-permissive phenotype.

Example 22. Generation of PRRSV-permissive BHK21 stable cell lines using pcDNA3.1D-humCD163v2

Parental Baby Hamster Kidney (BHK21) cells were obtained from Pfizer Inc. and were grown at 37 degrees C and 5% CO₂ in growth media consisting of Dulbecco's Modified Eagle Medium (DMEM, Invitrogen catalog number 11965) supplemented with 10% fetal bovine serum (FBS), 1mM sodium pyruvate, 2mM L-glutamine and antibiotics. Tissue culture wells (35 mm) containing approximately 1x10⁶ cells each were transfected with 2 micrograms per well of pcDNA3.1D-humCD163v2, in DMEM without FBS or antibiotics, using Lipofectamine 2000 (Invitrogen catalog number 11668-027) according to the manufacturer's instructions. After overnight incubation, cells were washed with PBS and removed from the substrate using Accutase (Innovative Cells Technologies, catalog number AT104) and diluted in growth medium containing Geneticin (G418 sulfate, Invitrogen catalog number 10131-027) at 1.0 milligram per ml and seeded into 96-well plates at various densities to ensure recovery of single cell clones after Geneticin selection. Throughout Geneticin selection, media was changed approximately every 3 to 5 days. After selection, wells containing single cell clones were expanded into duplicate 96-well plates and incubated until 100% confluency was achieved. One set of wells was screened for permissivity by infecting with PRRSV isolate P129, incubated for a minimum 48 hours. Seven candidate clones were found to be PRRSV-permissive. There was some evidence of phenotypic heterogeneity in each of the seven candidate clones, likely because they were not clonal. Therefore, the candidate clones were single-cell cloned by limiting dilution in G418 containing medium. One single cell clone with clear PRRS-permissivity was obtained and designated BHK-cDNA3.1D-humCD163v2-H9.

Example 23. Generation of PRRSV-permissive feline kidney stable cell lines using pcDNA3.1D-humCD163v2

Parental Norden Labs Feline Kidney (NLFK) cells were grown at 37 degrees C and 5% CO₂ in growth media consisting of Dulbecco's Modified Eagle Medium (DMEM, Invitrogen catalog number 11965) supplemented with 10% fetal bovine serum (FBS), 1mM sodium pyruvate, 2mM L-glutamine and antibiotics. Tissue culture wells (35 mm) containing approximately 1x10⁶ cells each were transfected with 2 micrograms per well of pcDNA3.1D-humCD163v2 in DMEM without FBS or antibiotics, using Lipofectamine 2000 (Invitrogen catalog number 11668-027) according to the manufacturer's instructions. After overnight incubation, cells were washed with PBS, removed from the substrate using Accutase (Innovative Cells Technologies, catalog number AT104), diluted in growth medium containing Geneticin (G418 sulfate, Invitrogen catalog number 10131-027) at 500 micrograms per ml, and seeded into 96-well plates at various densities to ensure recovery of single cell clones after Geneticin selection. Throughout Geneticin selection, media was changed approximately every 3 to 5 days. After selection, wells containing single cell clones were expanded into duplicate 96-well plates and incubated until 100% confluency was achieved. One set of wells was screened for PRRSV-permissivity by infecting with PRRSV isolate P129 for a minimum 48 hours. Five clones were found to be permissive. One of these, designated "FK-cDNA3.1D-humCD163v2-A6", clearly displayed the permissive phenotype (see Figure 13).

NLFK parent cells and one subclone of FK-cDNA3.1D-humCD163v2-A6 were examined for the CD163 expression. Cells were fixed in 80% acetone and reacted with Goat anti-human CD163 (R&D System at 1:200) for one hour following by washing with PBS. For visualization, donkey anti-Goat IgG conjugated with FITC (Bioscience Inc at 1:100) were used. No specific fluorescence was detected in the NLFK parent cells as shown in Figure 21A. The majority of the FK.A6.A2 subclone showed good fluorescent staining indicating the presence of CD163 (Figure 21B).

Example 24. Generation of PRRSV-permissive porcine kidney stable cell lines using pcDNA3.1D-humCD163v2

Parental Porcine Kidney (PK032495) cells were obtained from Pfizer Inc. and were grown at 37 degrees C and 5% CO₂ in growth media consisting of Dulbecco's Modified Eagle Medium (DMEM, Invitrogen catalog number 11965) supplemented with 5% fetal bovine serum (FBS), 1mM sodium pyruvate, 2mM L-glutamine and antibiotics. Tissue culture wells (35 mm) containing approximately 1x10⁶ cells each

were transfected with 2 micrograms per well of pcDNA3.1D-humCD163v2, in DMEM without FBS or antibiotics, using Lipofectamine 2000 (Invitrogen catalog number 11668-027) according to the manufacturer's instructions. After overnight incubation, cells were washed with PBS, removed from the substrate using Accutase
5 (Innovative Cells Technologies, catalog number AT104), diluted in growth medium containing Geneticin (G418 sulfate, Invitrogen catalog number 10131-027) at 1.0 milligram per ml, and seeded into 96-well plates at various densities to ensure recovery of single cell clones after Geneticin selection. Throughout Geneticin selection, media was changed approximately every 3 to 5 days. After selection, wells
10 containing single cell clones were expanded into duplicate 96-well plates and incubated until 100% confluency was achieved. One set of wells was screened for PRRSV-permissivity by infecting with PRRSV isolate P129 for a minimum 48 hours. Two clones were found to be permissive. One of these, designated "PK-cDNA3.1D-humCD163v2-B11" clearly showed the PRRSV-permissive phenotype.

15 **Example 25 Generation of PRRSV-permissive feline kidney stable cell line using ligated pRSV-Script MARC CD163v2**

A non-cloning based procedure to generate microgram quantities of linear DNA suitable for use in generating stable cell lines expressing CD163 from an RSV promoter was developed (Figure 4). A similar process was adapted to place simian
20 CD163v2 from MARC-145 cells behind the RSV promoter. The procedure involves the isolation and ligation of two pieces of DNA, one containing the neomycin gene and RSV promoter cassette derived from pRSV-script, and the other containing the MARC CD163v2 coding sequence from pCDNA3.1D MARC CD163v2. Vector plasmid pRSV-Script was linearized with *Hind* III and *Kpn* I. Plasmid was first
25 digested with *Kpn* I and was bunted with the Klenow fragment of *E. coli* DNA polymerase. This plasmid was then digested with *Hind* III immediately downstream of the RSV promoter. The pCDNA3.1D MARC CD163v2 clone was digested in the vector sequence downstream of the CD163 insert with *EcoRV*, and *Hind* III upstream of CD163. The CD163 coding sequence was liberated from the vector. For each
30 plasmid digestion the appropriate fragments were purified from agarose gels. A large-scale ligation reaction was performed as follows. Approximately 20 µg of each DNA fragment was incubated in a volume of 600 µL with 15 units of T4 DNA ligase. The reaction was incubated at room temperature for 1 hour. Following ligation, a linear

piece of DNA containing all of the appropriate elements was purified by agarose gel electrophoresis. Restriction enzyme digestion analysis was performed to confirm the authenticity of each ligated fragment. Ligation of the two DNA fragments via the cohesive *Hind* III termini resulted in the placement of the 5' sequences of the MARC
5 CD163 gene downstream of the RSV promoter, allowing for directed expression of CD163 in mammalian cells. Once isolated, the purified DNA was used to transfect selected mammalian cell lines.

Norden Labs Feline Kidney (NLFK) cells were grown at 37° C and 5% CO₂ in DMEM supplemented with 5% fetal bovine serum and antibiotics. NLFK cells were
10 seeded in 6 well plates at approximately 90% confluency and allowed to attach overnight. The cells were then transfected with ligated plasmid pRSV-MARC CD163v2 using Lipofectamine 2000 following the manufacturer's instructions. After 24 hours the cells were cloned as described in Example 12. Screening for PRRSV permissive cell clones was performed as described in Example 12. One clone was
15 positive for PRRSV infection and designated NLFK-MARC CD163 D4. This D4 clone has maintained the PRRSV permissive phenotype through 9 passages.

Example 26. Growth kinetics of PRRSV isolate NVSL 94-3 in recombinant BHK-21 and NLFK cells stably expressing susCD163v1 from the CMV promoter.

20 The amounts of progeny virus produced by PRRSV-infected BHK-21 or NLFK cells stably engineered to express susCD163v1 were quantitated. Four cell lines expressing susCD163v1, BHK/CMV/susv1 #3, BHK/CMV/susv1 #5, BHK/CMV/susv1 #12, and FK/CMV/susv1 G4 were seeded at sub confluency in 6 well plates and, after overnight incubation, were infected with the NVSL 94-3 isolate
25 of PRRSV. MARC-145 cells were included in the experiment for comparison. The cells were infected with virus at an m.o.i. of approximately 0.1. Virus was adsorbed for 60-90 minutes and was removed. Cells were washed three times with PBS to remove residual virus. One-milliliter aliquots were harvested from the cultures at 12-hour intervals starting immediately after infection and continued through 96 hrs.
30 Fresh culture media was added to the cells at various time points to maintain a culture volume sufficient to prevent the cell monolayer from drying out. Culture supernatants were stored at -80° until all samples were collected. The amount of PRRSV present in the culture supernatants was determined by plaque assay on MARC-145 cells. Figure

14 shows that all CD163 expressing recombinant cell lines tested were able to produce progeny PRRSV.

Example 27. Blocking PRRSV infection with anti-CD163 antibody: Transiently transfected cells.

5 BHK-21 cells, seeded in 24 well plates, were transiently transfected with the plasmid pCDNA3.1D-MARC-CD163v2 described in example 8, using Lipofectamine 2000 as described in example 14. After overnight incubation to allow expression of CD163, a titration of goat polyclonal antibody specific for human CD163 (R&D Systems, cat # AF1607) in PBS was added to the cells in a volume of 100µl. As a
10 control, equivalent amounts of normal goat IgG (R&D Systems, cat # AB-108-C) were used. Following a one-hour incubation at 37°C, the monolayers were infected with approximately 1×10^7 pfu of a recombinant P129 strain of PRRSV that expresses GFP. The cell monolayers, with anti-CD163 antibody and PRRSV, were incubated at 37°C for one hour at which time the virus inoculum/antibody mixture was aspirated,
15 the cell monolayer washed once with PBS, and 1ml of growth medium added to the wells. The cells were incubated for 24 hours at 37°C to allow PRRSV directed GFP expression. For analysis, the cells were trypsinized, resuspended in 500µl of PBS and analyzed by flow cytometry to innumerate the PRRSV infected cells via GFP expression. For flow cytometry, uninfected BHK-21 cells were used to set the
20 baseline for fluorescence detection, and approximately 100,000 cells were analyzed from each subsequent sample. The results of this analysis, shown in Figure 15, show that the CD163 specific antibody was able to significantly reduce the number of infected cells when compared to cells incubated with normal goat IgG.

Example 28: Blocking PRRSV infection by anti CD163 antibody: Stably transfected cells.

25 The NLFK cells that stably express human CD163 (FK-cDNA3.1D-humCD163v2-A6), described in Example 23, were seeded into 24 wells plates. After allowing the cells to attach overnight, a titration of goat polyclonal antibody specific for human CD163 (R&D Systems, cat # AF1607) in PBS was added to the cells in a
30 volume of 100µl. As a control, equivalent amounts of normal goat IgG (R&D Systems, cat # AB-108-C) were used. Following a one-hour incubation at 37°C, the monolayers were infected with approximately 1×10^7 pfu of a recombinant P129 strain of PRRSV that expresses GFP. The cell monolayers, with anti-CD163 antibody and

PRRSV, were incubated at 37°C for one hour at which time the virus inoculum/antibody mixture was aspirated, the cell monolayer washed once with PBS, and 1ml of growth medium added to the wells. The cells were incubated for 24 hours at 37°C to allow PRRSV directed GFP expression. For analysis, the cells were

5 trypsinized, resuspended in 500µl of PBS, and analyzed by flow cytometry to innumerate the PRRSV infected cells via GFP expression. Approximately 100,000 cells were analyzed from each sample. The results of this analysis, shown in Figure 16, show that the CD163 specific antibody was able to significantly reduce the number of infected cells when compared to cells incubated with normal goat IgG.

10 **Example 29. Generation of PRRSV-permissive porcine kidney stable cell lines using pRSV-susCD163v2.**

Porcine kidney cells (PK032495) were cultured as described in Example 21. For transfection, cells were seeded in a 24 well plate at 80% confluency and allowed to recover overnight. Transfection of ligated pRSV-susCD163v2 DNA described in

15 Example 5 was performed using Lipofectamine 2000 (Invitrogen) following the manufacturer's instructions. Subsequent cloning and selection of PRRSV permissive cells was performed essentially as described in Example 14. The initial cloning by limiting dilution failed to yield single cell derived clones, so 5 wells with PRRSV permissive cells were recloned by limiting dilution to yield clonal cell lines. 10 clones

20 were selected for further study and one of these clones, PK-RSVScript-susCD163v2 #9 showed the ability to support foci growth of PRRSV early after infection (see Figure 18).

Example 30. Generation of PRRSV-permissive feline kidney stable cell lines using pRSV-susCD163v2.

25 NLFK feline kidney cells were cultured as described in Example 17. For transfection, cells were seeded at approximately 80% of maximal density in 24 well plates. After overnight incubation the monolayers were transfected with ligation derived RSV/susCD163v2 (see example 5) using Lipofectamine following the manufacturer's instructions. Cloning of the transfected cells and selection of PRRSV

30 permissive cell clones was performed essentially as described in Example 14. Of the 67 cell clones tested for PRRSV permissivity, 20 were found to be positive. An example of the staining observed is shown in Figure 19.

Example 31. Passage of PRRSV isolate P201 in PK-RSVScript-susCD163v2 cells
Amplification of a PRRSV clinical isolate was performed as follows. Peripheral

35 alveolar macrophage (PAM) cells were seeded at 5.4E6 cells per 10cm² in a 6 well

dish using OptiMEM media supplemented with 2% FBS. After 6 hours the media was aspirated and a 2 ml aliquot of serum harvested from a PRRSV infected pig was added to the cells. Following a 90 minute adsorption, the serum inoculum was removed and replaced with OptiMEM. At approximately 40 post infection the
5 supernatant was harvested and clarified with a 10 minute centrifugation. The supernatant was directly used to infect PK-RSVScript-susCD163v2 clone #9 cells using a 6 hour adsorption. After removal of the inoculum the cells were refed with D-MEM. The P201 virus was serially passaged on the PK-RSVScript-susCD163v2 #9 cell line using alternating infected cell and cell free supernatant passes. We observed
10 that for efficient spread of the virus, the cells should be seeded at 50-70 % confluency the day before infection, using flasks of cells that were kept at sub-confluency. To follow the progression of infection, each passage was replicated in multiple wells of identically infected cells and at each day one of the wells was acetone fixed and stained with the FITC labeled monoclonal antibody SDOW17. If the percentage of
15 infected cells was not greater than 50% and significant progression of foci development over the prior days observations were not seen, the cells in an equivalent well were trypsinized and passed to multiple fresh wells. These infected cell passages were typically at a 1:4 split and sometimes included the addition of an equivalent number of cells from an uninfected culture. Alternatively, if the SDOW17 staining
20 revealed that the infected cell foci had spread sufficiently to account for greater than 50% of the total cells, cell free supernatant was harvested and used to infect multiple wells of freshly seeded cells (Figure20). After 11 passages the intervening cell passages were not necessary as the virus was able to grow to sufficient titer to allow consecutive cell free supernatant passaging of the virus.

25 **Example 32. Screening various CD163 cell lines for permissivity to various European and North American PRRSV isolates**

Various CD163 transgenic cell lines were assessed for permissivity to low passage European and North American PRRSV isolates (see Table 7). Transgenic CD163 cell lines as described in earlier examples included NLFK-MARC CD163 D4, PK-RSVScript-susCD163v2clone #9 and PK-CMV-susCD163v1-A10. Each CD163 cell
30 line along with cell lines MARC-145, parental feline kidney, parental porcine kidney cell lines (serving as controls) were planted onto 96-well tissue culture plates. Growth media was removed from monolayers and inoculated with 0.1 mL per well of each PRRSV isolate. At day 3 post infection, the plates were fixed with 80% acetone and

stained with FITC-conjugated monoclonal antibody SDOW17 (Rural Technologies Inc.) which is specific for the nucleocapsid. Results of the fluorescent antibody (FA) assay are in Table 7. **Table 7**

FA results of screening of various CD163 cell lines for permissivity to European and North American PRRSV isolates						
CD163 Cell Line	PRRSV Isolate ^a					
	EU98V226	P129	P201	1151	94-3	IND5
NLFK-MARC CD163 D4	++	+	+++	+++	++	++++
PK-RSVScript-susCD163v2clone #9	+	+	++	+	+	++
PK-CMV-susCD163v1-A10	+	+	++	++	++	++
MARC-145	++	+	+++	+	++++	+++
Porcine Kidney (parental)	-	-	-	-	-	-
Feline Kidney (parental)	-	-	-	-	-	-

5

^a All PRRSV isolates are North American except EU98V226 is a European isolate.

Example 33. Phorbol 12-myristate 13-acetate (PMA) induction of CD163 renders human U937 cells permissive to PRRSV infection.

10 Human U937 cells obtained from ATCC (CRL-1593.2) were propagated in RPMI medium containing serum and additives according to ATCC specifications. These cells are known to express CD163 when activated by PMA treatment (Gronlund et al., 2000). U937 cells were seeded in duplicate in wells of a 6-well plate. One set of wells was treated with 100 ng/ml of PMA and the other set was left
15 untreated. Three days after PMA stimulation, one well from each set was infected with the P129 isolate of PRRSV. The other well from each set was fixed and stained for expression of CD163 in an indirect immunofluorescent antibody assay using goat-anti human CD163 (R&D System) and donkey anti-goat IgG conjugated with FITC (BioDesign International).

20 Untreated U937 cells continued propagation to high density 3 days after initial planting. PMA-treated U937 cells stopped propagating, became enlarged, and attached to the surface of the culture wells. A small fraction of untreated U937 were positive for CD163 staining, whereas almost all PMA-treated U937 were positive for CD163 staining. In untreated U937 no PRRSV infected cells were observed.
25 However, hundreds of PMA treated U937 cells became infected by PRRSV. This demonstrates that non-permissive cells can be rendered permissive for PRRSV infection following chemical induction of CD163 expression.

64680-1737

Additional features and variations of the invention will be apparent to those skilled in the art from the entirety of this application, including the detailed description, and all such features are intended as aspects of the invention. Likewise, features of the invention described herein can be re-combined into additional embodiments that also are intended as aspects of the invention, irrespective of whether the combination of features is specifically mentioned above as an aspect or embodiment of the invention. Also, only such limitations that are described herein as critical to the invention should be viewed as such; variations of the invention lacking limitations that have not been described herein as critical are intended as aspects of the invention.

It will be clear that the invention may be practiced otherwise than as particularly described in the foregoing description and examples.

Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, are within the scope of the invention.

DEMANDES OU BREVETS VOLUMINEUX

**LA PRÉSENTE PARTIE DE CETTE DEMANDE OU CE BREVETS
COMPREND PLUS D'UN TOME.**

CECI EST LE TOME __1__ DE __2__

NOTE: Pour les tomes additionels, veuillez contacter le Bureau Canadien des Brevets.

JUMBO APPLICATIONS / PATENTS

**THIS SECTION OF THE APPLICATION / PATENT CONTAINS MORE
THAN ONE VOLUME.**

THIS IS VOLUME __1__ OF __2__

NOTE: For additional volumes please contact the Canadian Patent Office.

CLAIMS

1. A method of facilitating production of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) from a culture of vertebrate cells, comprising the steps of:
 - (a) providing a recombinant vertebrate cell transfected with an exogenous polynucleotide that encodes a mammalian CD163 polypeptide having a transmembrane domain and at least 70% sequence identity to SEQ ID NO:14, so that expression of CD163 polypeptide in said cell is increased compared to a non-transfected cell;
 - (b) contacting a culture of said cell with PRRSV virus under conditions which permit infection of the cells and growth of the virus; and
 - (c) recovering virus from said culture.
2. The method of claim 1, wherein the cell was previously PRRSV permissive and is rendered more PRRSV permissive than it was before the cell was infected with PRRSV virus.
3. The method of claim 1, wherein the cell did not previously express a CD163 polypeptide and is induced to express CD163.
4. The method of any one of claims 1 to 3, wherein the cell is a baby hamster kidney cell (BHK21), porcine kidney cell, feline kidney cell, avian cell, or swine testicular cell.
5. The method of any one of claims 1 to 4, wherein the PRRSV is of the European genotype.
6. The method of any one of claims 1 to 4, wherein the PRRSV is of the North American genotype.
7. The method of claim 1, wherein the polynucleotide encodes a polypeptide having a transmembrane domain and at least 90% sequence identity to SEQ ID NO:14.
8. The method of claim 1, wherein the polynucleotide encodes a polypeptide having a transmembrane domain and at least 90% sequence identity to SEQ ID NO:19.

9. The method of claim 1, wherein the polynucleotide encodes a polypeptide having a transmembrane domain and at least 97% sequence identity to SEQ ID NO:24.
10. The method of claim 1, wherein the polynucleotide encodes a polypeptide having a transmembrane domain and at least 90% sequence identity to SEQ ID NO:32.
11. The method of claim 1, wherein the polynucleotide encodes a polypeptide having a transmembrane domain and at least 90% sequence identity to SEQ ID NO:46.
12. The method of any one of claims 1 to 11, further comprising the step of producing a vaccine from the recovered PRRSV.
13. The method of claim 12, wherein the vaccine comprises inactivated PRRSV.
14. The method of claim 12, wherein the vaccine comprises live attenuated PRRSV.
15. The method of claim 1, wherein transfection with the exogenous polynucleotide that encodes the mammalian CD163 polypeptide is accomplished by electroporation.
16. The method of claim 1, wherein the exogenous polynucleotide used to transfect the recombinant vertebrate cell, encodes a fusion protein that comprises the mammalian CD163 polypeptide.
17. A method of facilitating production of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) from a culture of vertebrate cells, comprising the steps of:
 - (a) providing a recombinant vertebrate cell transfected with an exogenous polynucleotide that encodes a polypeptide that encodes a mammalian CD163 polypeptide having a transmembrane domain and at least 85% sequence identity to SEQ ID NO:2, so that expression of the polypeptide in said cell is increased compared to a non-transfected cell;
 - (b) contacting a culture of said cell with PRRSV virus under conditions which permit infection of the cells and growth of the virus; and
 - (c) recovering virus from said culture.

18. The method of claim 17, wherein the cell was previously PRRSV permissive and is rendered more PRRSV permissive than it was before the cell was infected with PRRSV virus.
19. The method of claim 17 or claim 18, wherein the cell is a baby hamster kidney cell (BHK21), porcine kidney cell, feline kidney cell, avian cell, or swine testicular cell.
20. The method of any one of claims 17 to 19, wherein the PRRSV is of the European genotype.
21. The method of any one of claims 17 to 19, wherein the PRRSV is of the North American genotype.
22. The method of claim 17, wherein the polynucleotide encodes a polypeptide having a transmembrane domain, and at least 90% sequence identity to SEQ ID NO:2.
23. The method of claim 17, wherein the polynucleotide encodes a polypeptide having a transmembrane domain and at least 95% sequence identity to SEQ ID NO:2.
24. The method of claim 17, wherein the polynucleotide encodes a polypeptide having a transmembrane domain and at least 99% sequence identity to SEQ ID NO:2.
25. The method of any one of claims 17 to 24, further comprising the step of producing a vaccine from the recovered PRRSV.
26. The method of claim 25, wherein the vaccine comprises inactivated PRRSV.
27. The method of claim 25, wherein the vaccine comprises live attenuated PRRSV.
28. The method of claim 17, wherein transfection with the exogenous polynucleotide is accomplished by electroporation.
29. The method of claim 17, wherein said exogenous polynucleotide used to transfect the recombinant vertebrate cell, encodes a fusion protein that comprises the mammalian CD163 polypeptide.

Figure 1

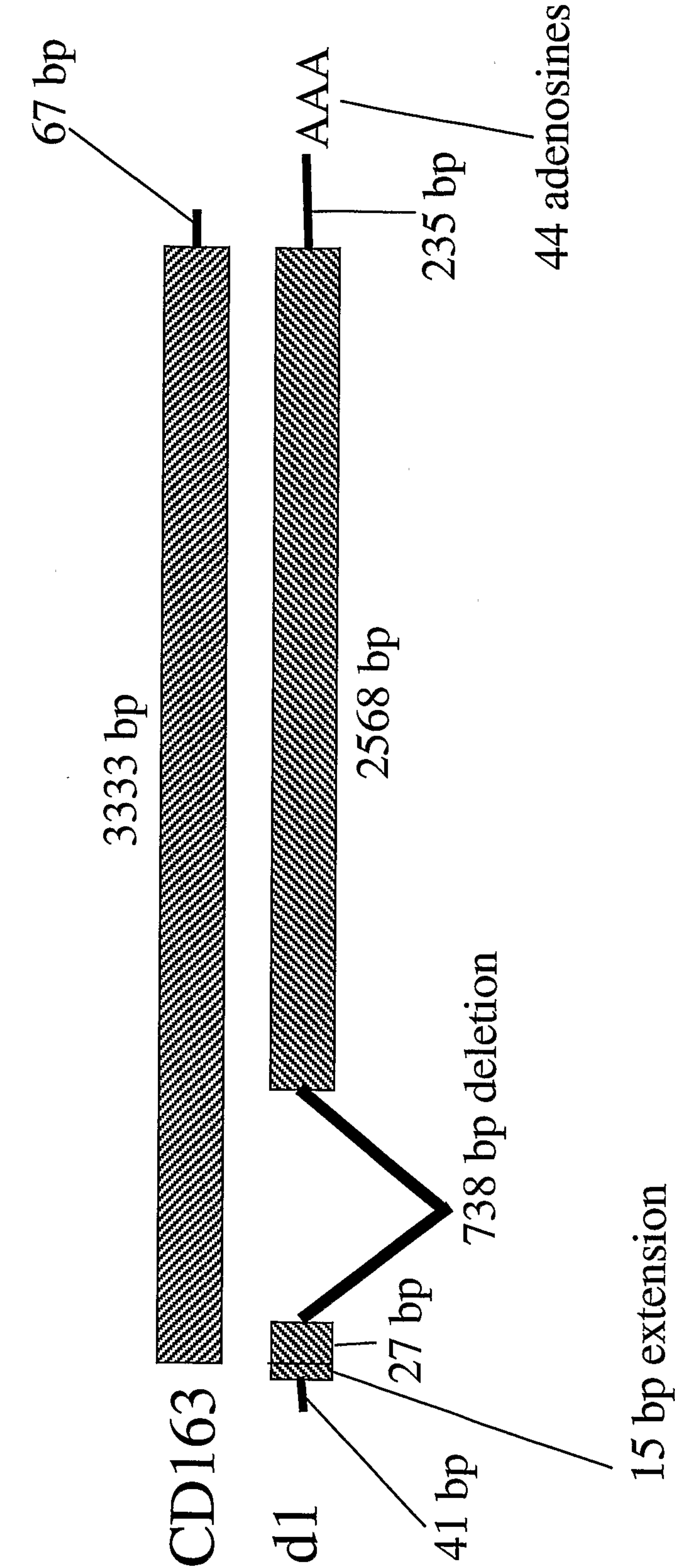


Figure 2A

AJ311716 (porcine) Pfizer_susCD163v1	1 MVLL ⁶⁰ EDSGSADFRRC ⁶⁰ SAHLSSFTFAVAVLSACLVTS ⁶⁰ SLGGKD ⁶⁰ KELRLT ⁶⁰ GGENKC ⁶⁰ MDKLRMV ⁶⁰ LHENS ⁶⁰ GSAD.....
AJ311716 (porcine) Pfizer_susCD163v1	61 SGRVEVKVQEEWGTVCNNGWMDVVS ¹²⁰ VVCRQLGCP ¹²⁰ TAIKATGWANFSAGSGRIWMDHVSC ¹²⁰
AJ311716 (porcine) Pfizer_susCD163v1	121 RGNESALWDCKHDGWGKHNC ¹⁸⁰ THQQDAGVTCSDGSDLEMRLVNGNRC ¹⁸⁰ LGRIEVKFQERWG ¹⁸⁰
AJ311716 (porcine) Pfizer_susCD163v1	181 TVCDNDFNINHASV ²⁴⁰ CKQLECGSAVSFSGSANFGE ²⁴⁰ SGPIWFDDLV ²⁴⁰ CNGNESALWNCKHE ²⁴⁰
AJ311716 (porcine) Pfizer_susCD163v1	241 GWGKHNC ³⁰⁰ DHAE ³⁰⁰ DAGVIC ³⁰⁰ LN ³⁰⁰ GAD ³⁰⁰ LKLRVVDGLTE ³⁰⁰ CSGRLEVKFQGEWGTICDDGWDSD ³⁰⁰ DAA ³⁰⁰ LKLRVVDGVTECSGRLEVKFQGEWGTICDDGWDSD ³⁰⁰ DAA ³⁰⁰
AJ311716 (porcine) Pfizer_susCD163v1	301 VACKQLGCPTAVTAIGRVNASEGTGHIW ³⁶⁰ LD ³⁶⁰ SVSCHGHESALWQCRHHEWGKHYCNHNEDA ³⁶⁰ VACKQLGCPTAVTAIGRVNASEGTGHIW ³⁶⁰ LD ³⁶⁰ SVSCHGHESALWQCRHHEWGKHYCNHNEDA ³⁶⁰
AJ311716 (porcine) Pfizer_susCD163v1	361 GVTCS ⁴²⁰ DGSDLELRLKGGSHCAGTVEVEIQKL ⁴²⁰ VGKVC ⁴²⁰ DRSWGLKEADV ⁴²⁰ VC ⁴²⁰ RLGCGSALK ⁴²⁰ GVTCS ⁴²⁰ DGSDLELRLKGGSHCAGTVEVEIQKL ⁴²⁰ VGKVC ⁴²⁰ DRSWGLKEADV ⁴²⁰ VC ⁴²⁰ RLGCGSALK ⁴²⁰
AJ311716 (porcine) Pfizer_susCD163v1	421 TSYQVYSKTKATNTWLFVSSCNGNETSLWDCKNWQWGGLSCDHYDEAKITCSAHRKPRLV ⁴⁸⁰ TSYQVYSKTKATNTWLFVSSCNGNETSLWDCKNWQWGGLSCDHYDEAKITCSAHRKPRLV ⁴⁸⁰
AJ311716 (porcine) Pfizer_susCD163v1	481 GGDIPCSGRVEVQHGD ⁵⁴⁰ TWGTVCDSDFSLEAASVLCRELQC ⁵⁴⁰ GT ⁵⁴⁰ V ⁵⁴⁰ SL ⁵⁴⁰ LGGAH ⁵⁴⁰ FGEGSGQIW ⁵⁴⁰ GGDIPCSGRVEVQHGD ⁵⁴⁰ TWGTVCDSDFSLEAASVLCRELQC ⁵⁴⁰ GT ⁵⁴⁰ V ⁵⁴⁰ SL ⁵⁴⁰ LGGAH ⁵⁴⁰ FGEGSGQIW ⁵⁴⁰

Figure 2B

AJ311716 (porcine)	541	600
Pfizer_susCD163v1	AEFFQCEGHESHL ^{SL} CPVAPRPDGTCSHSRDVG ^V VC ^S RYTQIRLVNGKTPCEGRVELNIL	
	AEFFQCEGHESHL ^{SL} CPVAPRPDGTCSHSRDVG ^V VC ^S RYTQIRLVNGKTPCEGRVELNIL	
AJ311716 (porcine)	601	660
Pfizer_susCD163v1	GSWGS ^{LC} NSHWD ^{ME} DAHVL ^C QQLK ^{CG} VAL ^{SI} PGGAPFGK ^{GE} QV ^{RR} HM ^{FH} CTGTEK ^{HM} GD	
	GSWGS ^{LC} NSHWD ^{ME} DAHVL ^C QQLK ^{CG} VAL ^{SI} PGGAPFGK ^{GE} QV ^{RR} HM ^{FH} CTGTEK ^{HM} GD	
AJ311716 (porcine)	661	720
Pfizer_susCD163v1	CSV ^T ALGAS ^{LC} SSGQ ^V ASV ^{IC} SGN ^Q SQ ^{TL} SPCN ^{SS} SSDP ^{SS} SI ^I SEESG ^V ACIGSGQ ^L RL	
	CSV ^T ALGAS ^{LC} SSGQ ^V ASV ^{IC} SGN ^Q SQ ^{TL} SPCN ^{SS} SSDP ^{SS} SI ^I SEEN ^G VACIGSGQ ^L RL	
AJ311716 (porcine)	721	780
Pfizer_susCD163v1	VDGGRCAGRVEV ^{YP} GASWGT ^{IC} DDSWDL ND AHV ^V CK ^{QL} SCGWA ^{IN} ATGSAHFGE ^{GT} GPI	
	VDGGRCAGRVEV ^Y <u>HEG</u> SWGT ^{IC} DDSWDL ND AHV ^V CK ^{QL} SCGWA ^{IN} ATGSAHFGE ^{GT} GPI	
AJ311716 (porcine)	781	840
Pfizer_susCD163v1	WLDEIN ^C NGKESH ^I WQCHSHG ^{WR} HNCR ^{HK} EDAG ^{VI} CSEF ^{MS} LRL ^{IS} ENSRET ^C AGR ^{LE} V	
	WLDEIN ^C NGKESH ^I WQCHSHG ^{WR} HNCR ^{HK} EDAG ^{VI} CSEF ^{MS} LRL ^{IS} ENSRET ^C AGR ^{LE} V	
AJ311716 (porcine)	841	900
Pfizer_susCD163v1	FYNGAWGSVGRNSMSPATVGV ^V CRQLGCADR ^{GD} ISPASSDK ^{TV} SRHM ^V VDNVQCPKGP ^{DT}	
	FYNGAWGSV ^G <u>K</u> NSMSPATVGV ^V CRQLGCADR ^{GD} ISPASSDK ^{TV} SRHM ^V VDNVQCPKGP ^{DT}	
AJ311716 (porcine)	901	960
Pfizer_susCD163v1	LWQCPSSPWKKRLASPSEETW ^{IT} CANKIRLQEGNT ^{NC} SGRVEIWYGGSWG ^{TV} CDDSWD ^{LE}	
	LWQCPSSPWKKRLASPSEETW ^{IT} CANKIRLQEGNT ^{NC} SGRVEIWYGGSWG ^{TV} CDDSWD ^{LE}	
AJ311716 (porcine)	961	1020
Pfizer_susCD163v1	DAQV ^V CRQLGCGSAL ^E AGKEP ^{AF} GQGTG ^{PI} WLNEV ^K CKNEP ^{SL} WDCPARSWGHSDC ^{GHK}	
	DAQV ^V CRQLGCGSAL ^E AGKEA ^{AF} <u>G</u> QGTG ^{PI} WLNEV ^K CKNEP ^{SL} WDCPARSWGHSDC ^{GHK}	
AJ311716 (porcine)	1021	1080
Pfizer_susCD163v1	EDAAV ^T CSEIAKSRESL ^H ATGRSS ^F VALAIFGVILLAC ^L IAFLIWTQRRRQ ^R RLSVFSG	
	EDAAV ^T CSEIAKSRESL ^H ATGRSS ^F VALAIFGVILLAC ^L IAFLIWTQRRRQ ^R RLSVFSG	
AJ311716 (porcine)	1081	1116
Pfizer_susCD163v1	GENSVHQIQYREMN ^{SL} KADET ^{ML} NP ^{SG} DHSEVQ.	
	GENSVHQIQYREMN ^{SL} KADET ^{ML} NP ^{SG} DHSEVQ.	

Figure 3A

Martinez/Needleman-Wunsch DNA Alignment
Minimum Match: 9; Gap Penalty: 1.10; Gap Length Penalty: 0.33

Genbank AJ3111716 porcine CD163 cDNA
Insert from plasmid pSport-susCD163d1

-----GTAATAATACAAGAAGATTTAAATGGGCATAAAACTTGGAATGGACAAACTCAGA
ATGGTGCTACTTGAAGACTCTGGATCTGCAGACTTTAGAAGATGTTCTGCCCATTTAAAGTTCCTTCACTTTTGGCTGTAGTCGCTGTTCTCAGTGCCTGCT
ATGGTGCTACATGAA¹AACTCTGGATCT-----
TGGTCACTAGTTCTCTTGGAGGAAAGACAAGGAGCTGAGGCTAACGGGTGGTGAAACAAGTGCTCTGGAAGAGTGGAGGTGAAAGTGCAGGAGGAGTG

GGGAACTGTGTGTAATAATGGCTGGGACATGGATGTGGTCTCTGTGTTGTAGGCAGCTGGGATGTCCAACTGCTATCAAAGCCACTGGATGGGCTAAT

TTTAGTGCAGGTTCTGGACGCATTTGGATGGATCATGTTTCTTGTCTGAGGGAATCAGTCAGTCTCTTGGGACTGCAAAACATGATGGATGGGGAAGCATA

ACTGTACTCACCAACAGGATGCTGGAGTAACCTGCTCAGATGGATCTGATTTAGAGATGAGGCTGGTGAATGGAGGAAACCGGTGCTTAGGAAGAATAGA

AGTCAAATTTCAAGAGCGGTGGGAAACAGTGTGTGATGATAACTTCAACATAAAATCATGCTTCTGTGGTTTGTAAACAACTTGAATGTGGAAGTGTGTC

AGTTTCTCTGGTTCAGCTAAATTTTGGAGAAGGTTCTGGACCAATCTGGTTTGATGATCTTGTATGCAATGGAAATGAGTCAGTCTCTTGGAACCTGCAAAC

ATGAAGGATGGGAAAGCACAATTGGCATCATGTGAGGATGCTGGAGTGATTTGCTTAAATGGAGCAGACCTGAAACTGAGAGTGGTAGATGGACTCAC
-----GCAGACCTGAAACTGAGAGTGGTAGATGGAGTCCAC

TGAATGTTTCAAGGAGATTGGAAGTGAAATTTCCAAGGAGAATGGGGAACAATCTGTGATGATGGCTGGGATAGTGAATGCCGCTGTGGCATGTAAAGCAA
TGAATGTTTCAAGGAGATTGGAAGTGAAATTTCCAAGGAGAATGGGGAACAATCTGTGATGATGGCTGGGATAGTGAATGCCGCTGTGGCATGTAAAGCAA

CTGGGATGTC²CAACTGCTGTCACTGCCATTGGTCGAGTTAACGCCAGTGAAGGAACTGGACACATTTGGCTTGACAGTGTCTTGTGCCATGGACACGAGT
CTGGGATGTC³CAACTGCTGTCACTGCCATTGGTCGAGTTAACGCCAGTGAAGGAACTGGACACATTTGGCTTGACAGTGTCTTGTGCCATGGACACGAGT

CTGCTCTCTGGCAGTGTAGACACCATGAATGGGGAAGCATTATTGCAATCATAAATGAAGATGCTGGTGTGACATGTTCTGATGGATCAGATCTGGAACT
CTGCTCTCTGGCAGTGTAGACACCATGAATGGGGAAGCATTATTGCAATCATAAATGAAGATGCTGGTGTGACATGTTCTGATGGATCAGATCTGGAACT

Figure 3B

GAGACTTAAAGGTGGAGGCAGCCACTGTGCTGGACAGTGGAGGTGGAATTCAGAAACTGGTAGGAAAAAGTGTGATAGAAAGCTGGGGACTGAAAGAA
GAGACTTAAAGGTGGAGGCAGCCACTGTGCTGGACAGTGGAGGTGGAATTCAGAAAACCTGGTAGGAAAAAGTGTGATAGAAAGCTGGGGACTGAAAGAA

GCTGATGTGGTTTTCAGGCAGCTGGGATCTGCACCTCAAAACATCATATCAAGTTTATTTCCAAAAACCAAGGCACAAAAACACATGGCTGTTTGTAA
GCTGATGTGGTTTTCAGGCAGCTGGGATCTGCACCTCAAAACATCATATCAAGTTTATTTCCAAAAACCAAGGCACAAAAACACATGGCTGTTTGTAA

GCAGCTGTAATGGAAATGAAACTTCTCTTTTGGGACTGCAAGAAATTGGCAGTGGGTGGACTTAGTTGTGATCACTATGACGAAAGCCAAAAATTACCTGCTC
GCAGCTGTAATGGAAATGAAACTTCTCTTTTGGGACTGCAAGAAATTGGCAGTGGGTGGACTTAGTTGTGATCACTATGACGAAAGCCAAAAATTACCTGCTC

AGCCACACAGGAAACCCAGGCTGGTTGGAGGGGACATTCCTTGCTCTGGTCGTGTTGAAAGTACAAACATGGAGACACGTGGGGCACCCGTCTGTGATTCGTGAC
AGCCACACAGGAAACCCAGGCTGGTTGGAGGGGACATTCCTTGCTCTGGTCGTGTTGAAAGTACAAACATGGAGACACGTGGGGCACCCGTCTGTGATTCGTGAC

TTCTCTCTGGAGGCGGCCAGCGTGTGTGAGGGAACTACAGTGGGGCACTGTGGTTTCCCTCCTGGGGGAGCTCACTTTGGAGAAAGGAAGTGGACAGA
TTCTCTCTGGAGGCGGCCAGCGTGTGTGAGGGAACTACAGTGGGGCACTGTGGTTTCCCTCCTGGGGGAGCTCACTTTGGAGAAAGGAAGTGGACAGA

TCTGGGCTGAAGAAATTCAGTGTGAGGGGCACGAGTCCACCTTTCACCTCTGCCAGTAGCACCCGCCCTGACGGGACATGTAGCCACAGCAGGGACGT
TCTGGGCTGAAGAAATTCAGTGTGAGGGGCACGAGTCCACCTTTCACCTCTGCCAGTAGCACCCGCCCTGACGGGACATGTAGCCACAGCAGGGACGT

CGGCGTAGTCTGCTCAAGATACACAAAAATCCGCTTGGTGAATGGCAAGACCCCATGTGAAGGAAGAGTGGAGCTCAACATTCCTTGGGTCTTGGGGGTCC
CGGCGTAGTCTGCTCAAGATACACAAAAATCCGCTTGGTGAATGGCAAGACCCCATGTGAAGGAAGAGTGGAGCTCAACATTCCTTGGGTCTTGGGGGTCC

CTCTGCAACTCTCACTGGGACATGGAAGATGCCCATGTTTATGCCAGCAGCTTAAATGTGGAAGTTGCCCTTCTATCCGGGAGGAGCACCTTTTGGGA
CTCTGCAACTCTCACTGGGACATGGAAGATGCCCATGTTTATGCCAGCAGCTTAAATGTGGAAGTTGCCCTTCTATCCGGGAGGAGCACCTTTTGGGA

AAGGAAGTGAGCAGGTCTGGAGGCACATGTTTCACCTGCACTGGGACTGAGAAGCACATGGGAGATTGTTCCGTCACTGCTCTGGGCGCATCACTCTGTTC
AAGGAAGTGAGCAGGTCTGGAGGCACATGTTTTCACCTGCACTGGGACTGAGAAGCACATGGGAGATTGTTCCGTCACTGCTCTGGGCGCATCACTCTGTTC

TTCAGGGCAAGTGGCCTCTGTAATCTGCTCAGGGAACAGAGTCAGACACTATCCCCGTGCAATTTCATCATCTCGGACCCCATCAAGCTCTATTATTTC
TTCAGGGCAAGTGGCCTCTGTAATCTGCTCAGGGAACAGAGTCAGACACTATCCCCGTGCAATTTCATCATCTCGGACCCATCAAGCTCTATTATTTC

GAAAGAAAGTGGTGTGCTGCTGATAGGGAGTGGTCAACTTCGCTGGTCGATGGAGGTGGTCGTTGTGCTGGGAGAGTAGAGGTCTATCCTGGGGCATCCT
GAAAGAAAGTGGTGTGCTGCTGATAGGGAGTGGTCAACTTCGCTGGTCGATGGAGGTGGTCGTTGTGCTGGGAGAGTAGAGGTCTATCAATGAGGGCTCCT

GGGGCACCATCTGTGATGACAGCTGGGACCTGAATGATGCCCATGTGGTGTGCAACAGCTGAGCTGTGGATGGGCCATTAAATGCCACTGGTTCGTCTCA
GGGGCACCATCTGTGATGACAGCTGGGACCTGAATGATGCCCATGTGGTGTGCAACAGCTGAGCTGTGGATGGGCCATTAAATGCCACTGGTTCGTCTCA

TTTTTGGGGAAGGAACAGGGCCCATTTGGCTGGATGAGATAAACTGTAATGGAAAAAGAACTCATATTTGGCAATGCCACTCACATGGTTGGGGCGGCAC
TTTTTGGGGAAGGAACAGGGCCCATTTGGCTGGATGAGATAAACTGTAATGGAAAAAGAACTCATATTTGGCAATGCCACTCACATGGTTGGGGCGGCAC

Figure 3C

AAATTGCAGGCATAAGGAGGATGCAGGAGTCAATCTGCTCAGAGTTCAATGTCCTCTGAGACTGATCAGTGAAAAACAGCAGAGAGACCTGTGTGCAGGGCGCCTGG
AAATTGCAGGCATAAGGAGGATGCAGGAGTCAATCTGCTCGGAGTTCAATGTCCTCTCAGACTGATCAGTGAAAAACAGCAGAGAGACCTGTGTGCAGGGCGCCTGG
AAAGTTTTTTTACAACGGAGCTTTGGGGCAGCGTTGGCAGGAATAGCATGTCTCCAGCCACAGTGGGGTGGTATGCAGGCAGCTGGGCTGTGCAGACAGAGG
AAAGTTTTTTTACAACGGAGCTTTGGGGCAGCGTTGGCAAGAATAGCATGTCTCCAGCCACAGTGGGGTGGTATGCAGGCAGCTGGGCTGTGCAGACAGAGG
GGACATCAGCCCTGCATCTTCAGACAAGACAGTGTCCAGGCACATGTGGTGGACAATGTTTCAGTGTCTTAAAGGACCTGACACACTATGGCAGTGCCCC
GGACATCAGCCCTGCATCTTCAGACAAGACAGTGTCCAGGCACATGTGGTGGACAATGTTTCAGTGTCTTAAAGGACCTGACACACCTATATGGCAGTGCCCCA
TCATCTCCATGGAAGAAGAGACTTGGCCAGCCCCCTCAGAGGAGACATGGATCACATGTGCCAACAAAATAAGACTTCAAGAAGGAAAAACAATAATTGTTCTG
TCATCTCCATGGAAGAAGAGACTTGGCCAGCCCCCTCAGAGGAGACATGGATCACATGTGCCAACAAAATAAGACTTCAAGAAGGAAAAACAATAATTGTTCTG
GACGTGTGGAGATCTGGTACGGAGGTTCCCTGGGGCACTGTGTGTGACGACTCCTTGGGACCTTGAAGATGCTCAGGTGGTGTGCCGACAGCTGGGCTGTGG
GACGTGTGGAGATCTGGTACGGAGGTTCCCTGGGGCACTGTGTGTGACGACTCCTTGGGACCTTGAAGATGCTCAGGTGGTGTGCCGACAGCTGGGCTGTGG
CTCAGCTTTTGGAGGCAGGAAAAAGAGCCCGCATTTGGCCAGGGGACTTGGGCCCATATGGCTCAATGAAGTGAACTGCAAGGGGAATGAACCCCTCCTTGTGG
CTCAGCTTTTGGAGGCAGGAAAAAGAGCCCGCATTTGGCCAGGGGACTTGGGCCCATATGGCTCAATGAAGTGAACTGCAAGGGGAATGAAACCCTCCTTGTGG
GATTGTCTGTGCCAGATCCTGGGGCCACAGTGACTGTGGACACAAAGGAGGATGCTGTGTGACGTGTCAGAAAATTGCAAAGAGCCGAGAAATCCCTACATG
GATTGTCTGTGCCAGATCCTGGGGCCACAGTGACTGTGGACACAAAGGAGGATGCTGTGTGACGTGTTCAGAAAATTGCAAAGAGCCGAGAAATCCCTACATG
CCACAGGTCGCTCAATCTTTTGTGCACTTGTGCAATCTTTGGGGTCATTTCTGTGGCCTGTCTCATCGCATTCCTCAATTTGGACTCAGAAAGCGAAGACAGAG
CCACAGGTCGCTCAATCTTTTGTGCACTTGTGCAATCTTTGGGGTCATTTCTGTGGCCTGTCTCATCGCATTCCTCAATTTGGACTCAGAAAGCGAAGACAGAG
GCAGCGGCTCTCAGTTTTTCTCAGGAGGAGAGAAATCTGTCCATCAAATTCAAATACCGGGAGATGAATTTCTTGCCCTGAAAGCAGATGAAACGGATATGCTA
GCAGCGGCTCTCAGTTTTTCTCAGGAGGAGAGAAATCTGTCCATCAAATTCAAATACCGGGAGATGAATTTCTTGCCCTGAAAGCAGATGAAACGGATATGCTA
AATCCCTCAGGAGACCACTCTGAAGTACAATGAAAAGGAAAAATGGGAATTATAACCTGGTGAGTTTCAGCCTTTAAGATACCTTGTATGAAGACCTTGGACTA
AATCCCTCAGGAGACCACTCTGAAGTACAATGAAAAGGAAAAATGGGAATTATAACCTGGTGAGTTTCAGCCTTTAAGATACCTTGTATGAAGACCTTGGACTA

TTGAATGAGCAAGAATCTGCCCTTTACACTGAAGATTACAATACAGTCCTCTGTCTCCTGGTATTTCCAAAGACTGCTGTTGAATTTCTAAAAAaTAGATT

GGTGAATGTGACTACTCAAAGTTGTATGTAAAGACTTTCAAGGGCATTAATAAAAAAGAAATATGTCTGAAAAAaAAAAAAAAAAAAAAAAAAAA

AAAAAAAAAAAAA

Figure 4

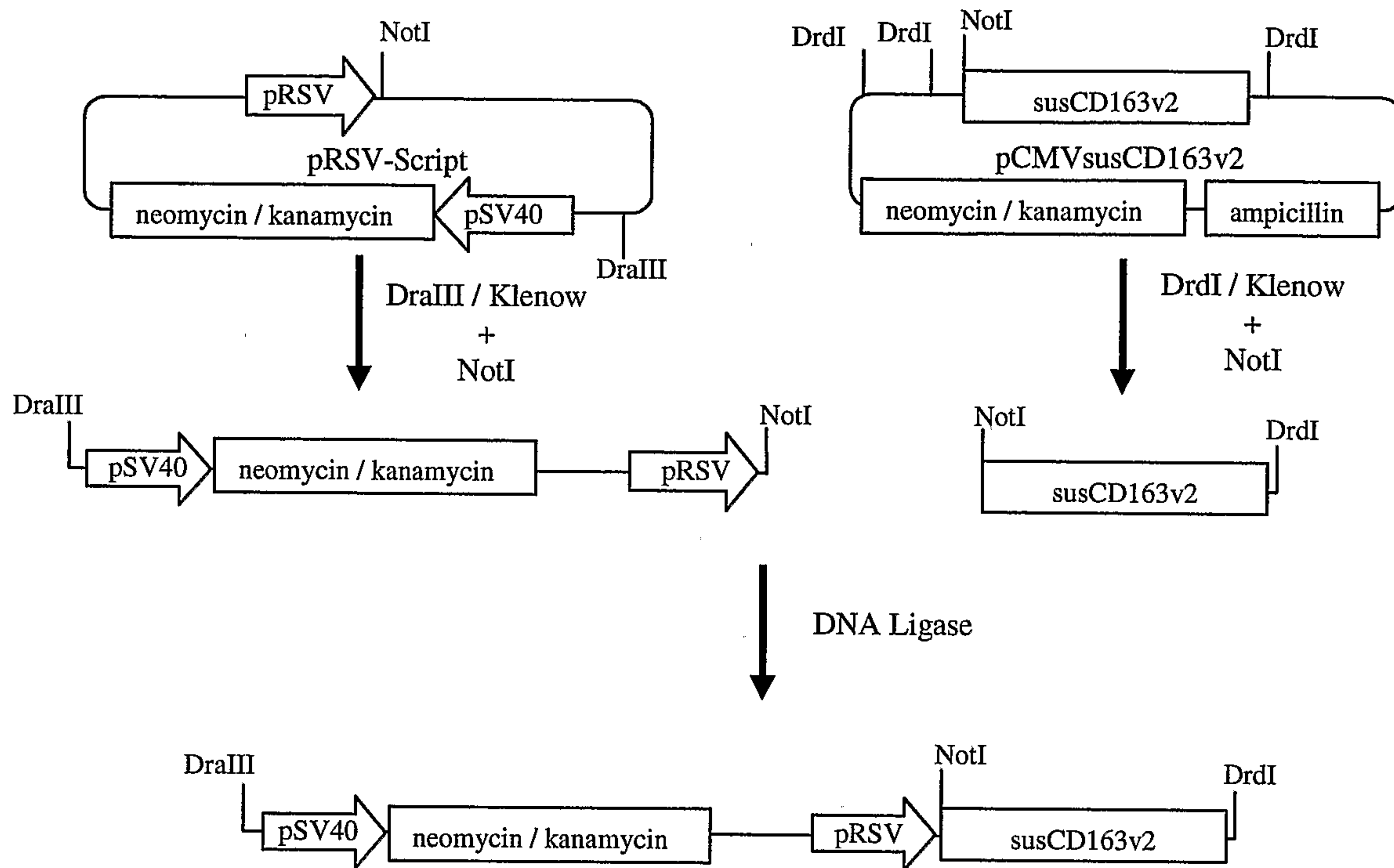
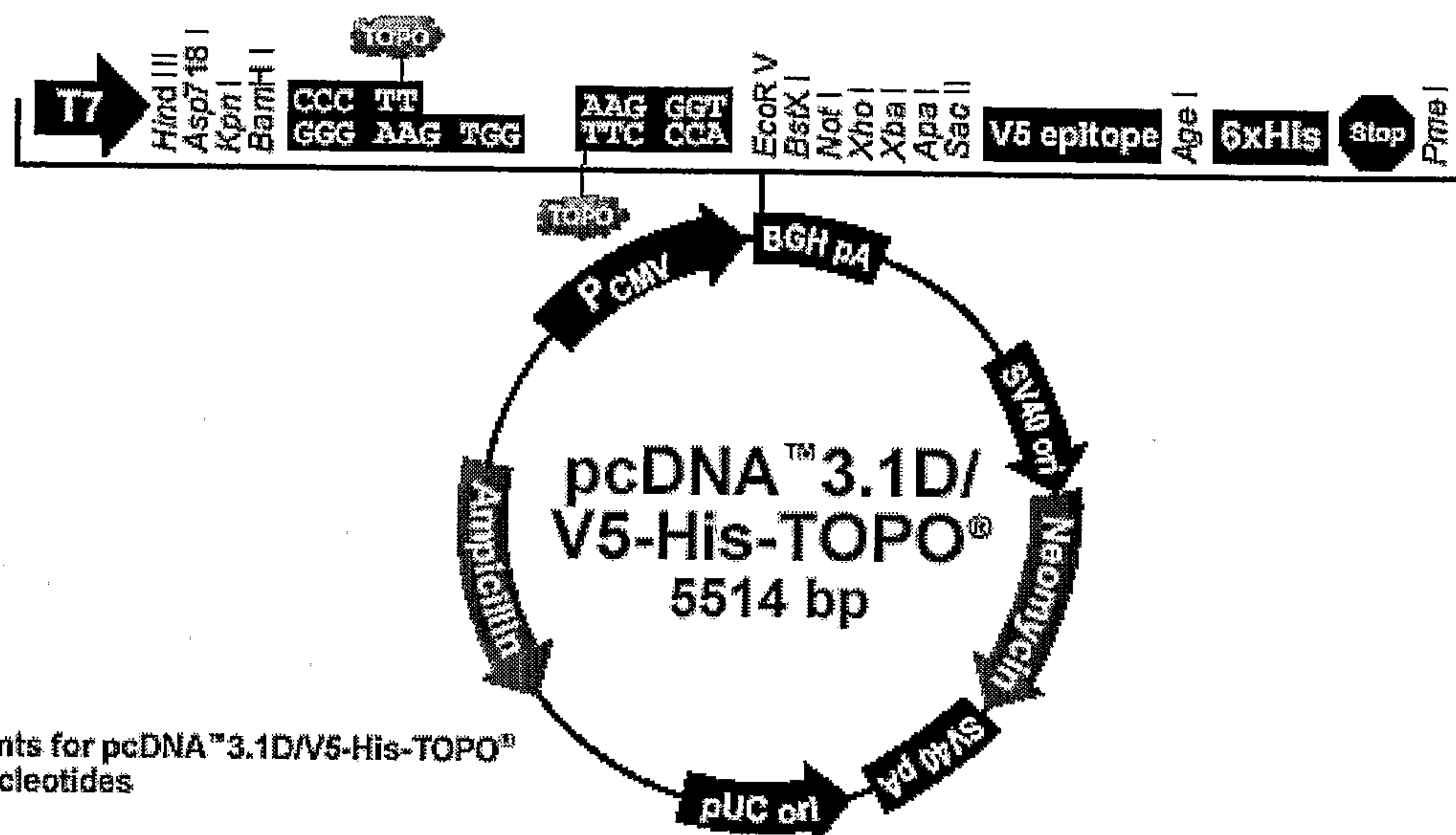


Figure 5



Comments for pcDNA[™]3.1D/V5-His-TOPO[®]
5514 nucleotides

CMV promoter: bases 232-819

T7 promoter/priming site: bases 863-882

TOPO[®] recognition site 1: bases 930-934

Overhang sequence (complementary strand): bases 935-938

TOPO[®] recognition site 2: bases 939-943

V5 epitope: bases 1011-1052

Polyhistidine (6xHis) tag: bases 1062-1079

BGH reverse priming site: bases 1102-1119

BGH polyadenylation signal: bases 1108-1332

SV40 early promoter and origin: bases 1833-2142

Neomycin resistance gene: bases 2217-3011

SV40 early polyadenylation signal: bases 3189-3319

pUC origin: bases 3700-4373 (complementary strand)

Ampicillin (*bla*) resistance gene: bases 4518-5378 (complementary strand)

bla promoter: bases 5379-5477 (complementary strand)

Figure 6

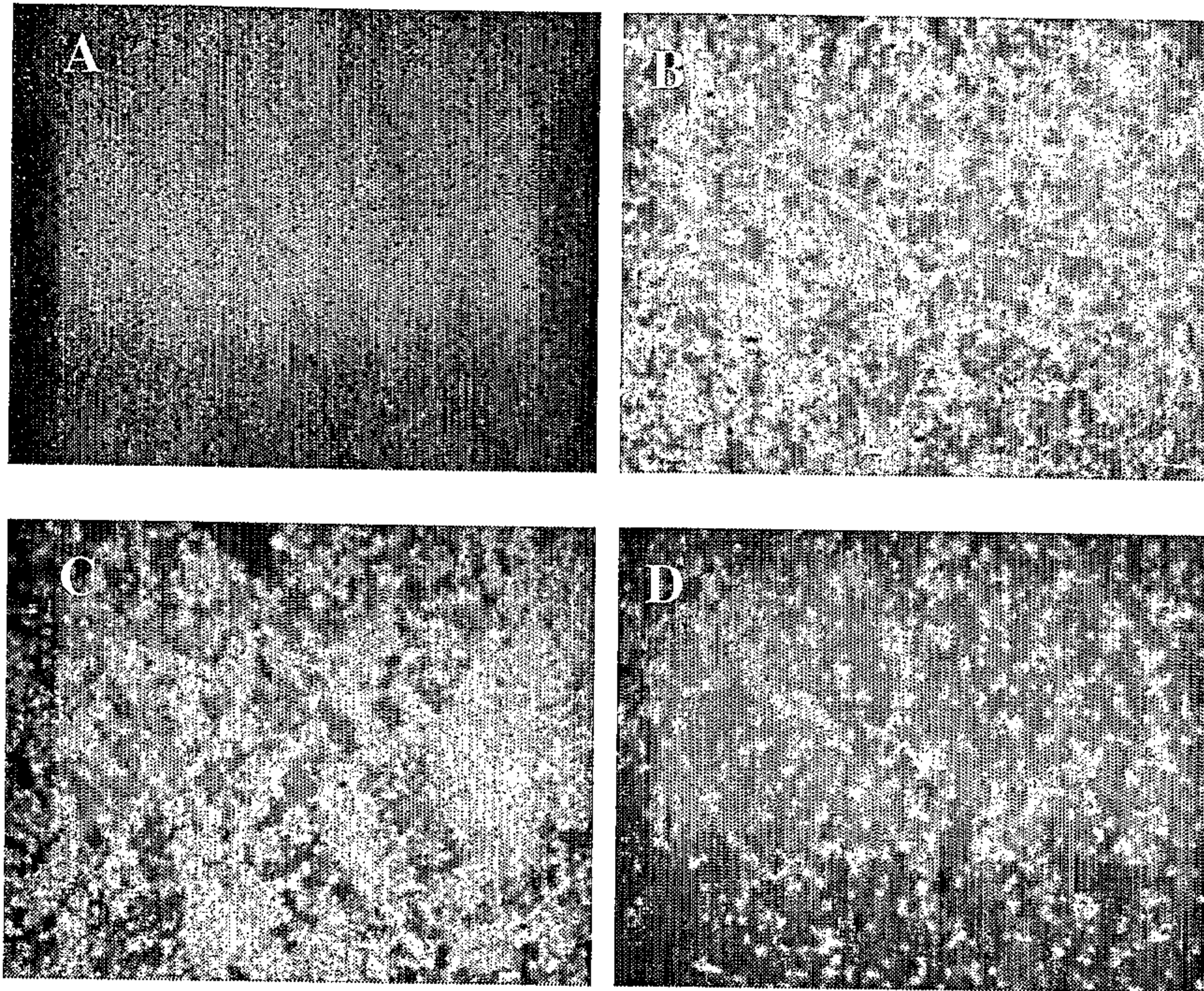


Figure 7

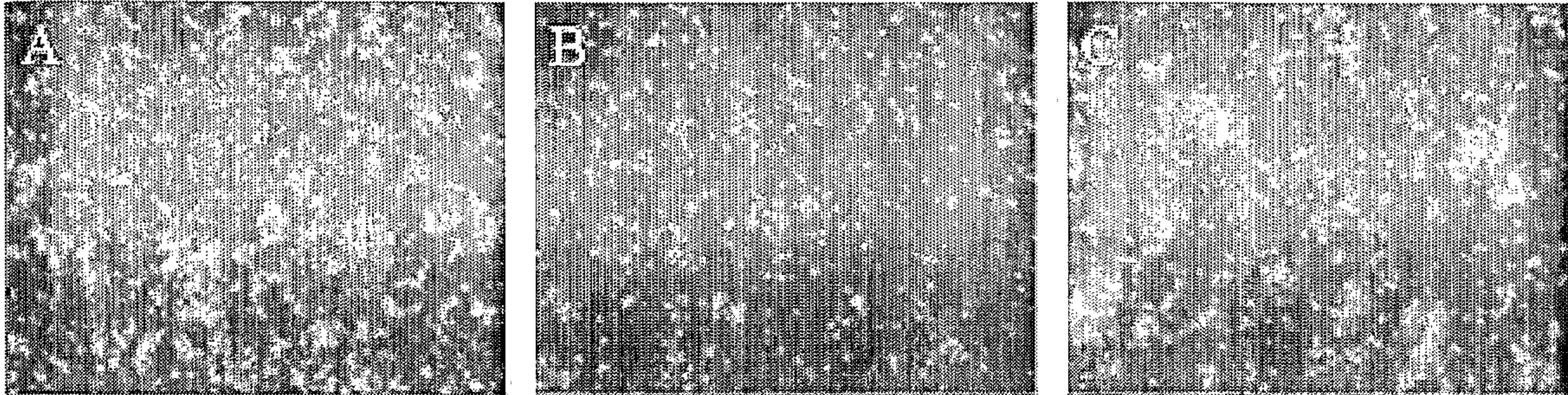


Figure 8

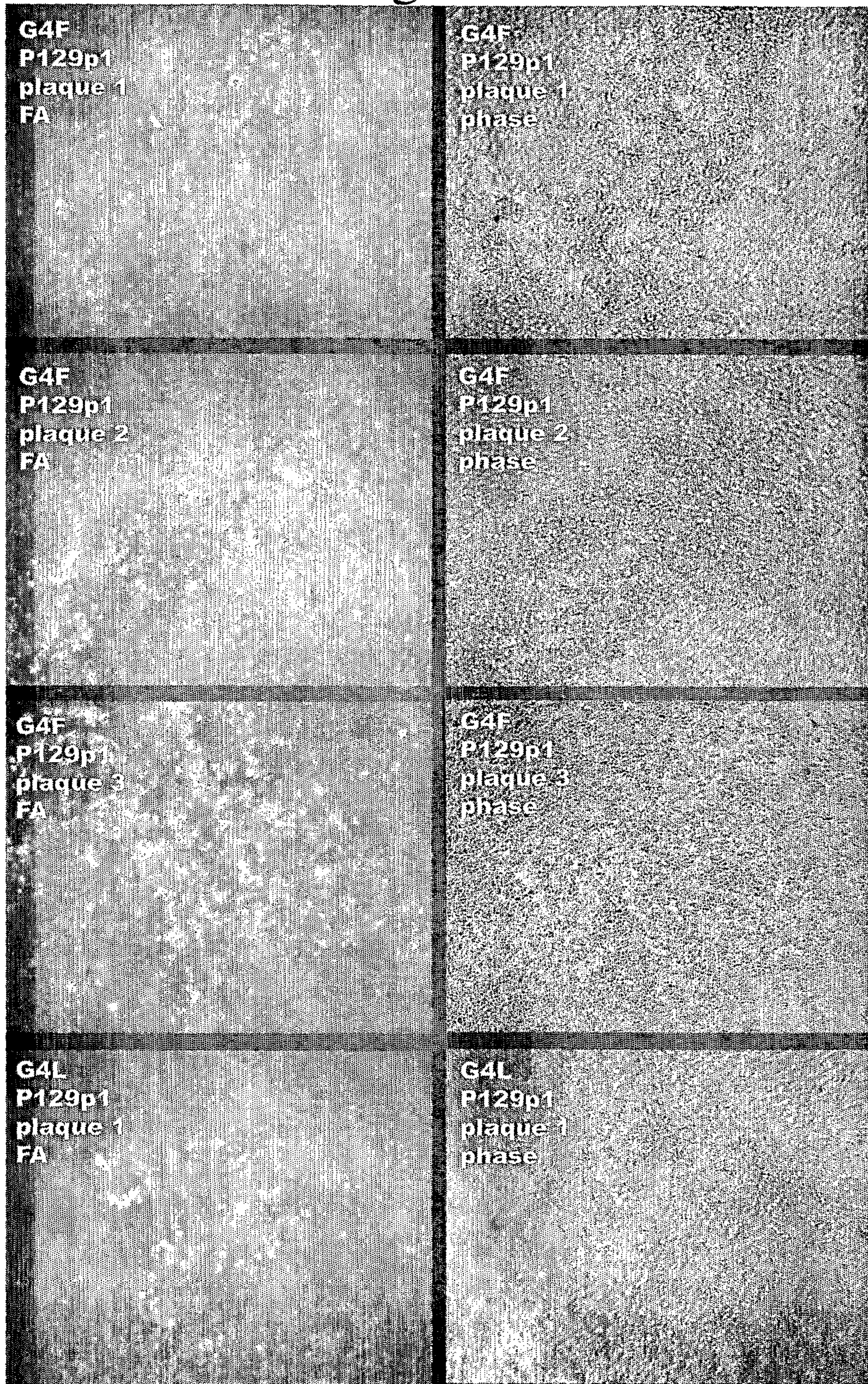


Figure 9

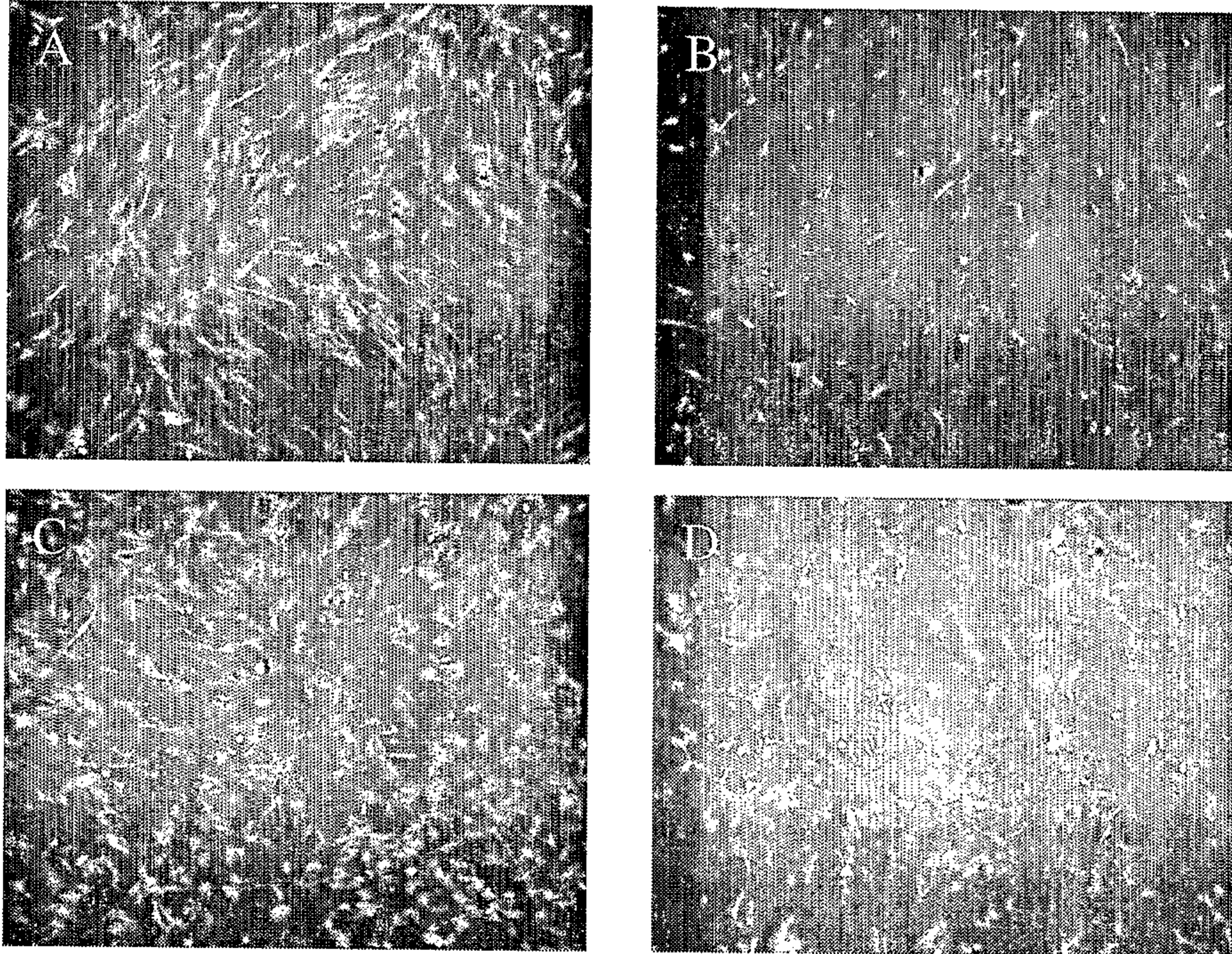


Figure 10

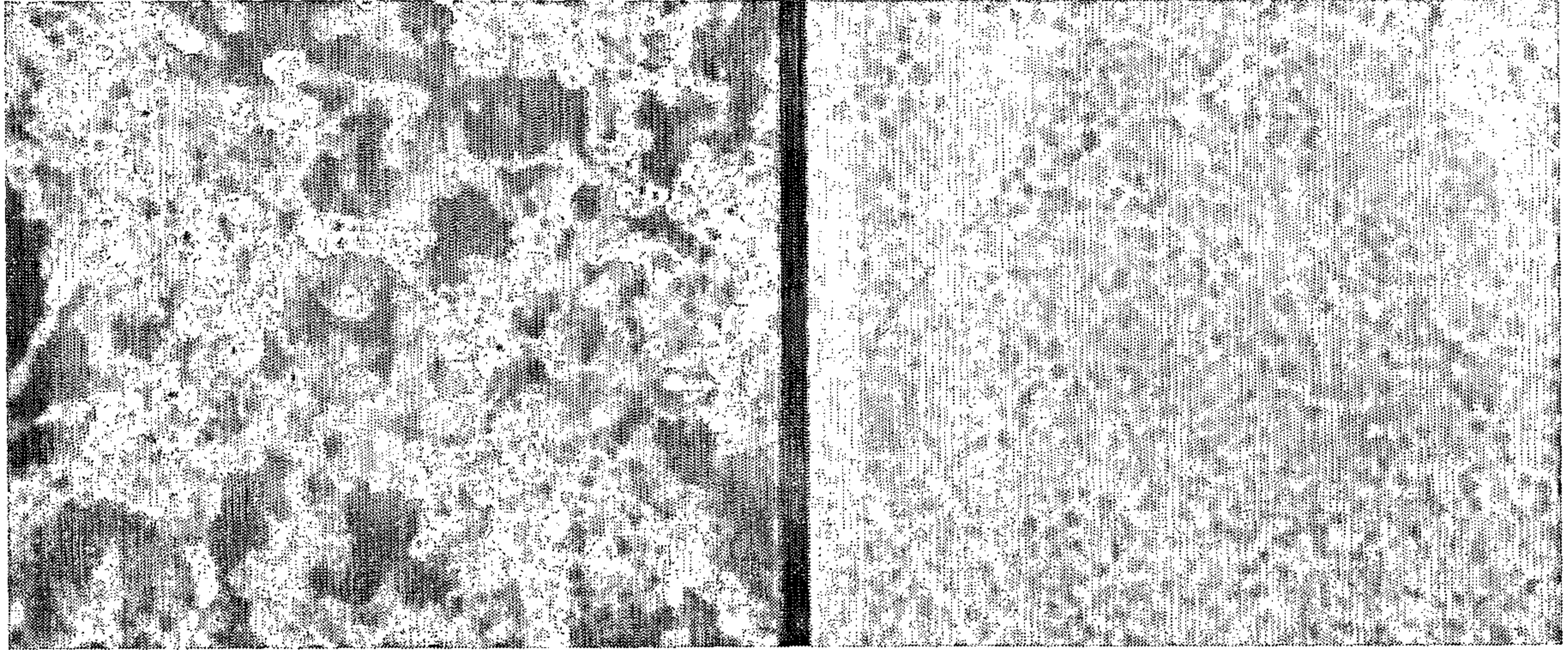


Figure 11

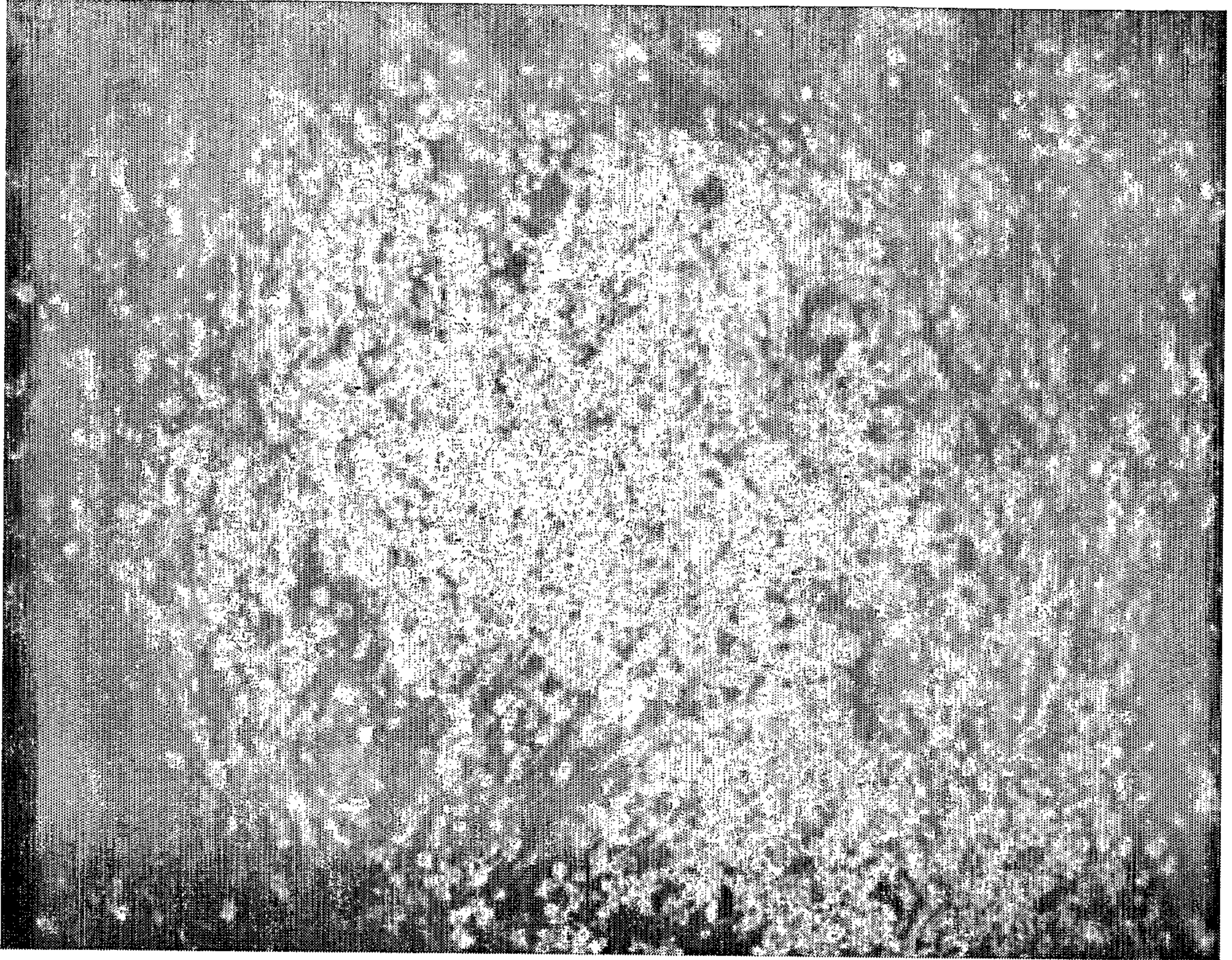


Figure 12

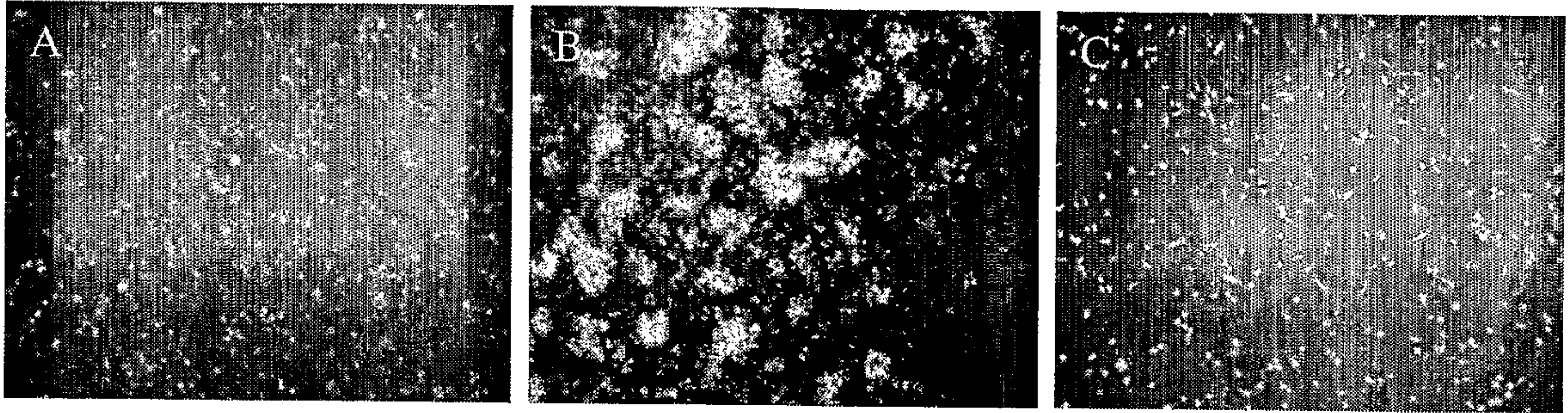


Figure 13

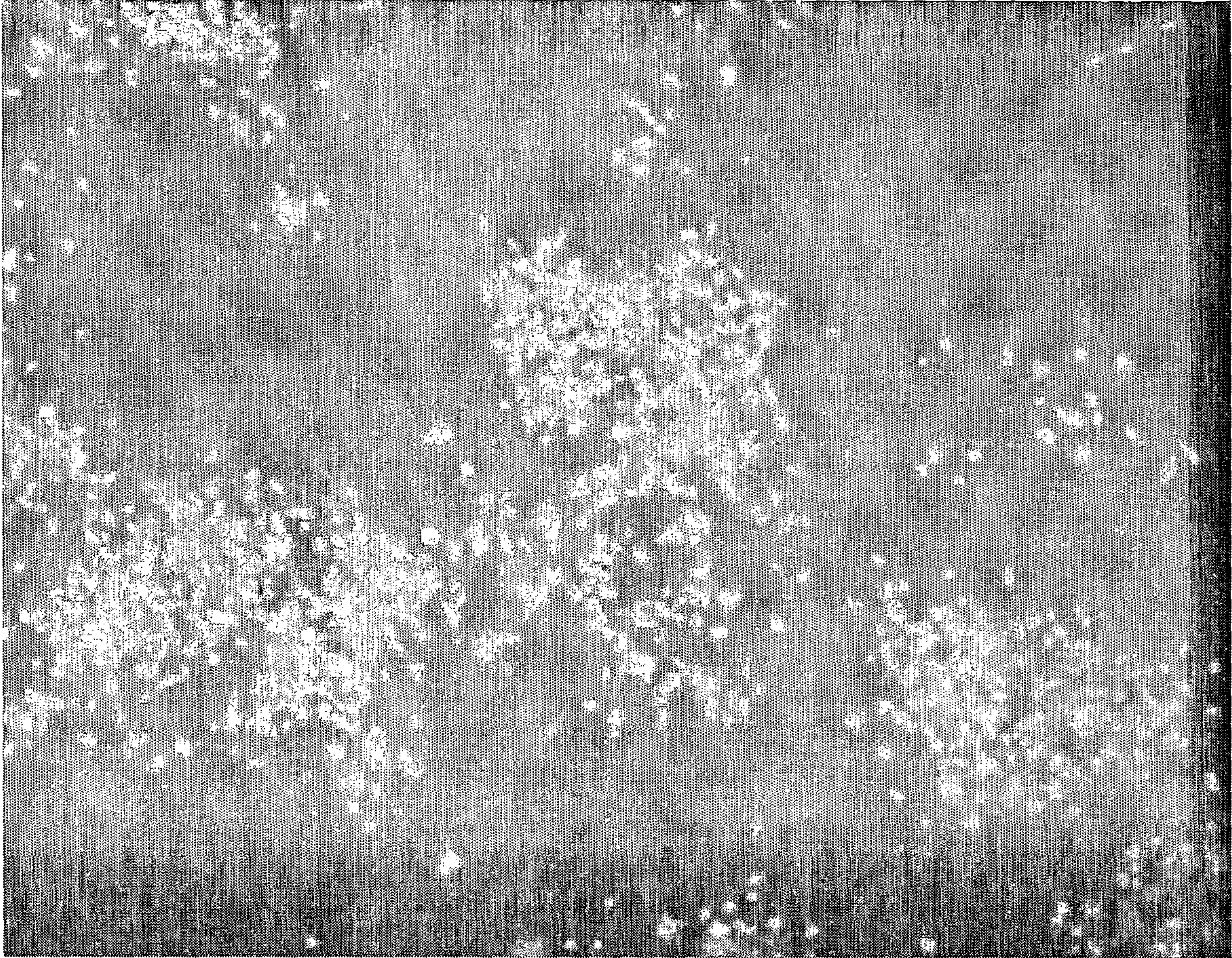


Figure 14

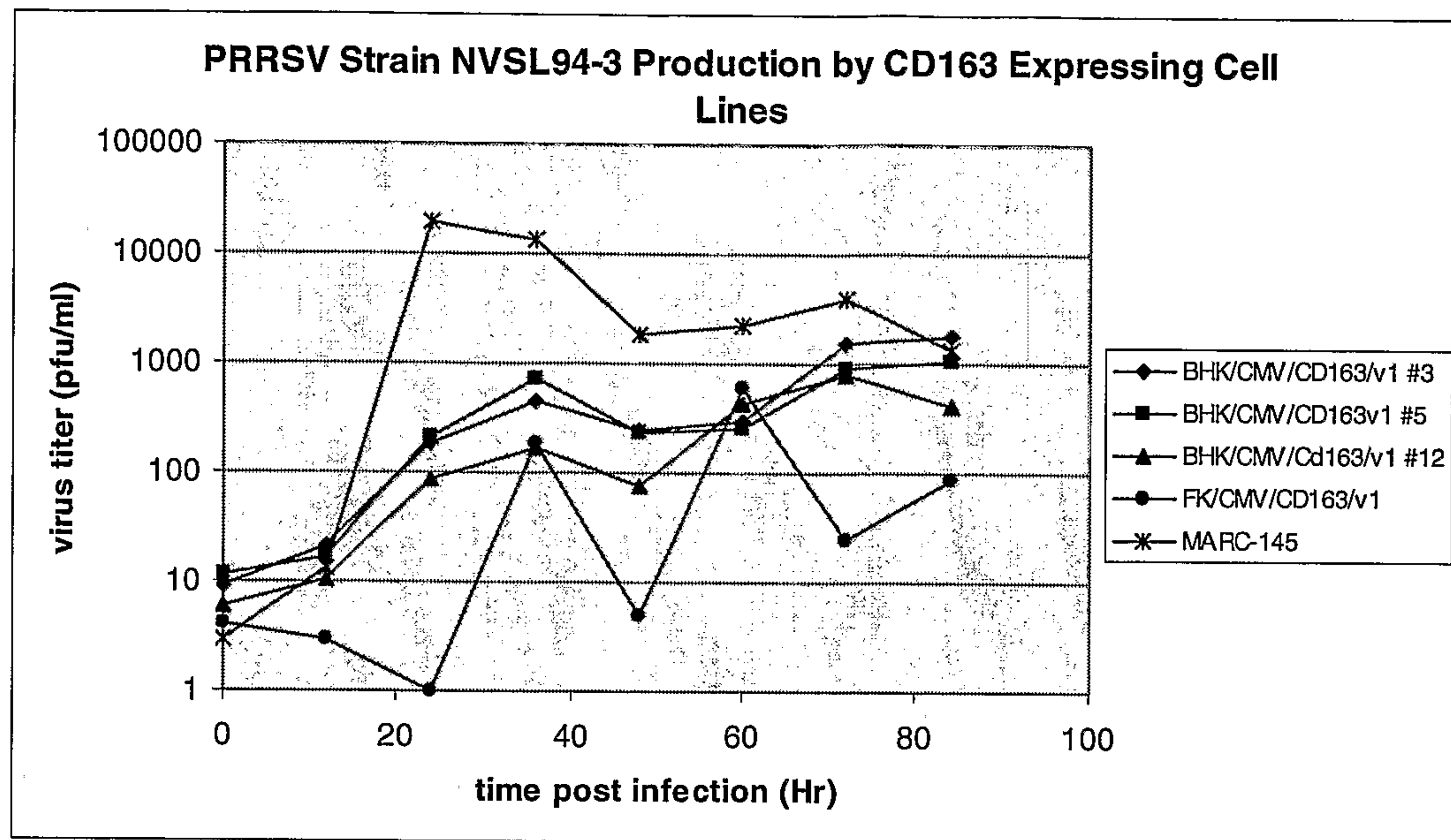


Figure 15

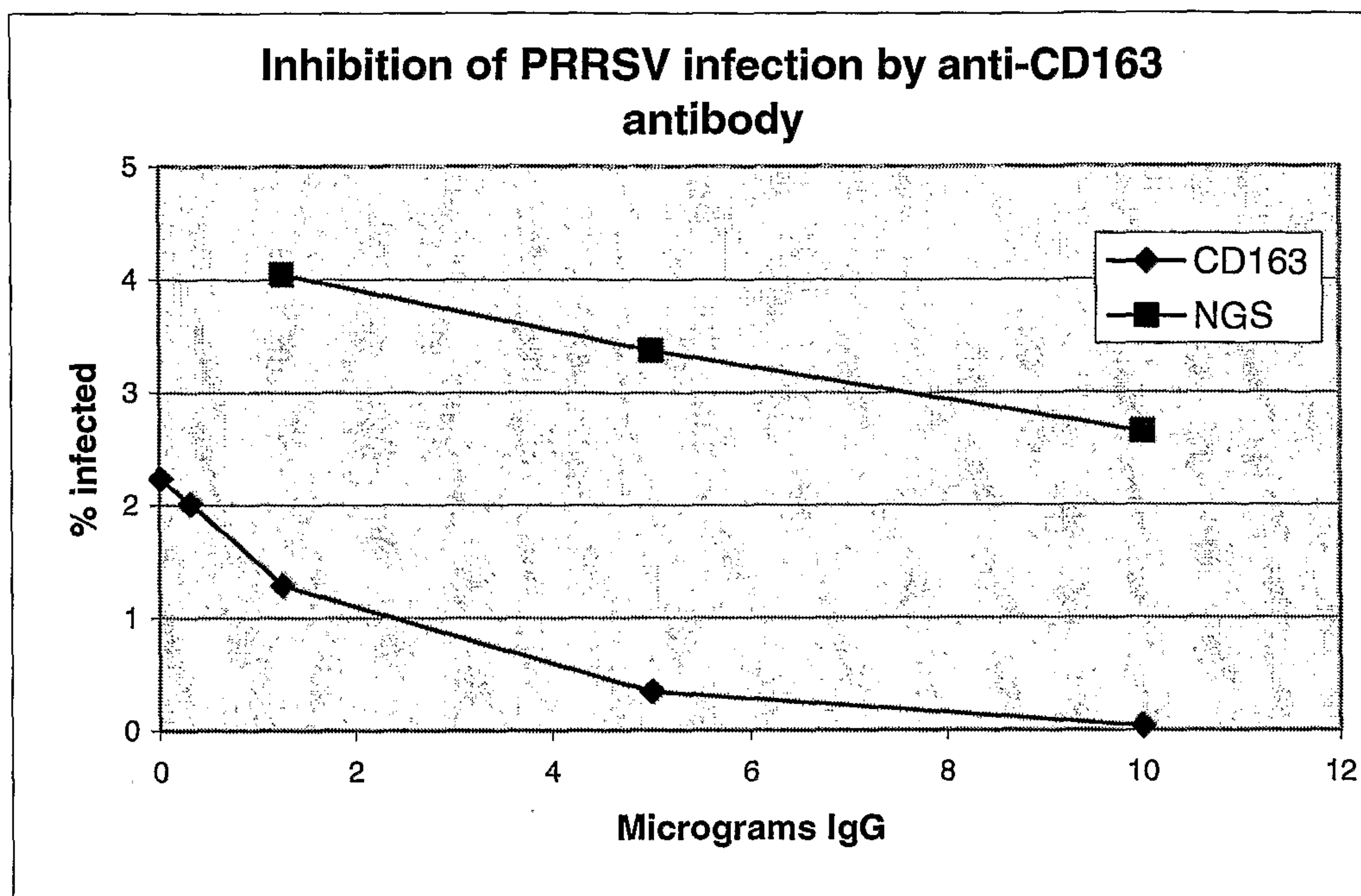


Figure 16

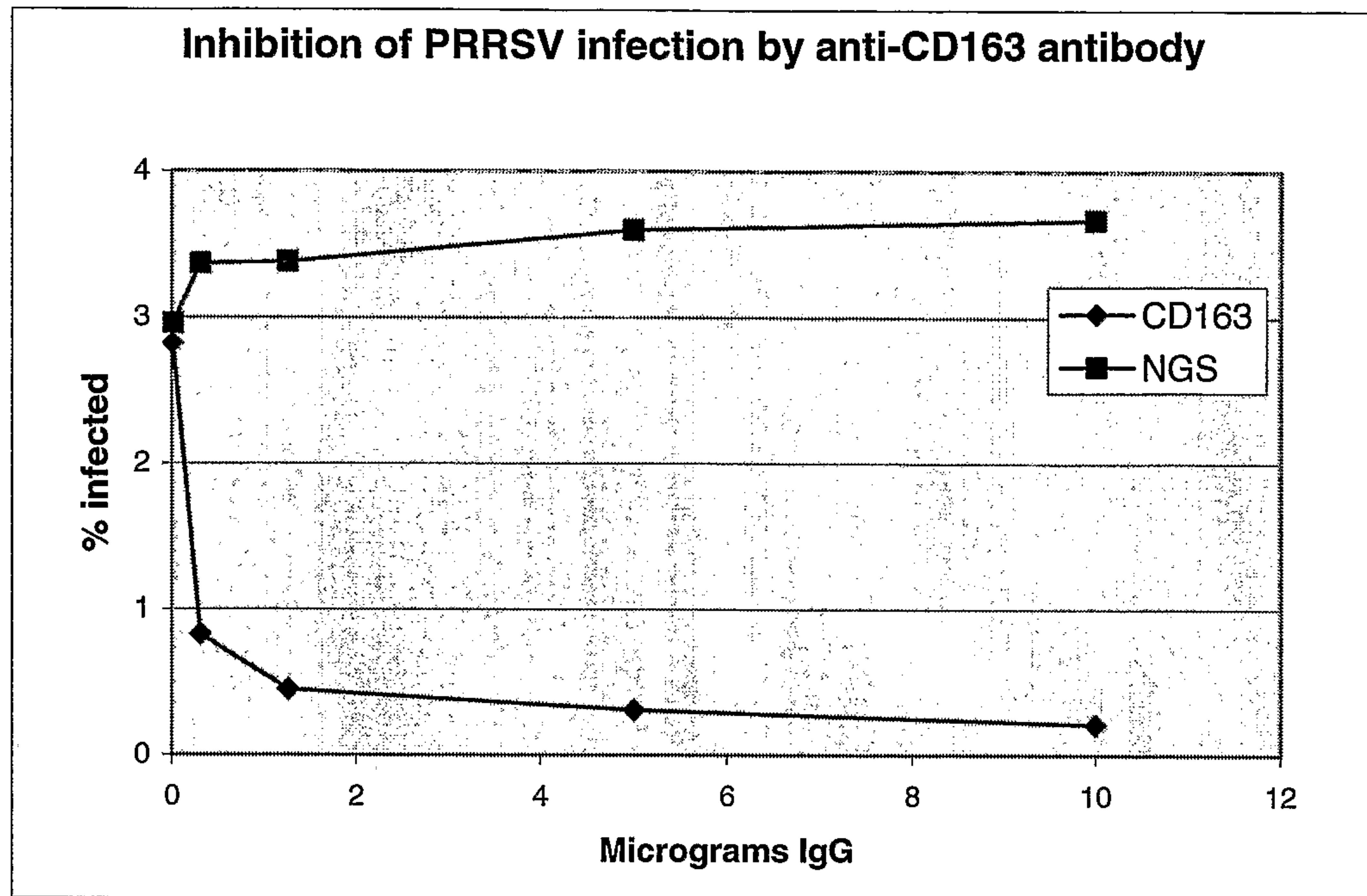


Figure 17

Splice variants of CD163 from Vero cells

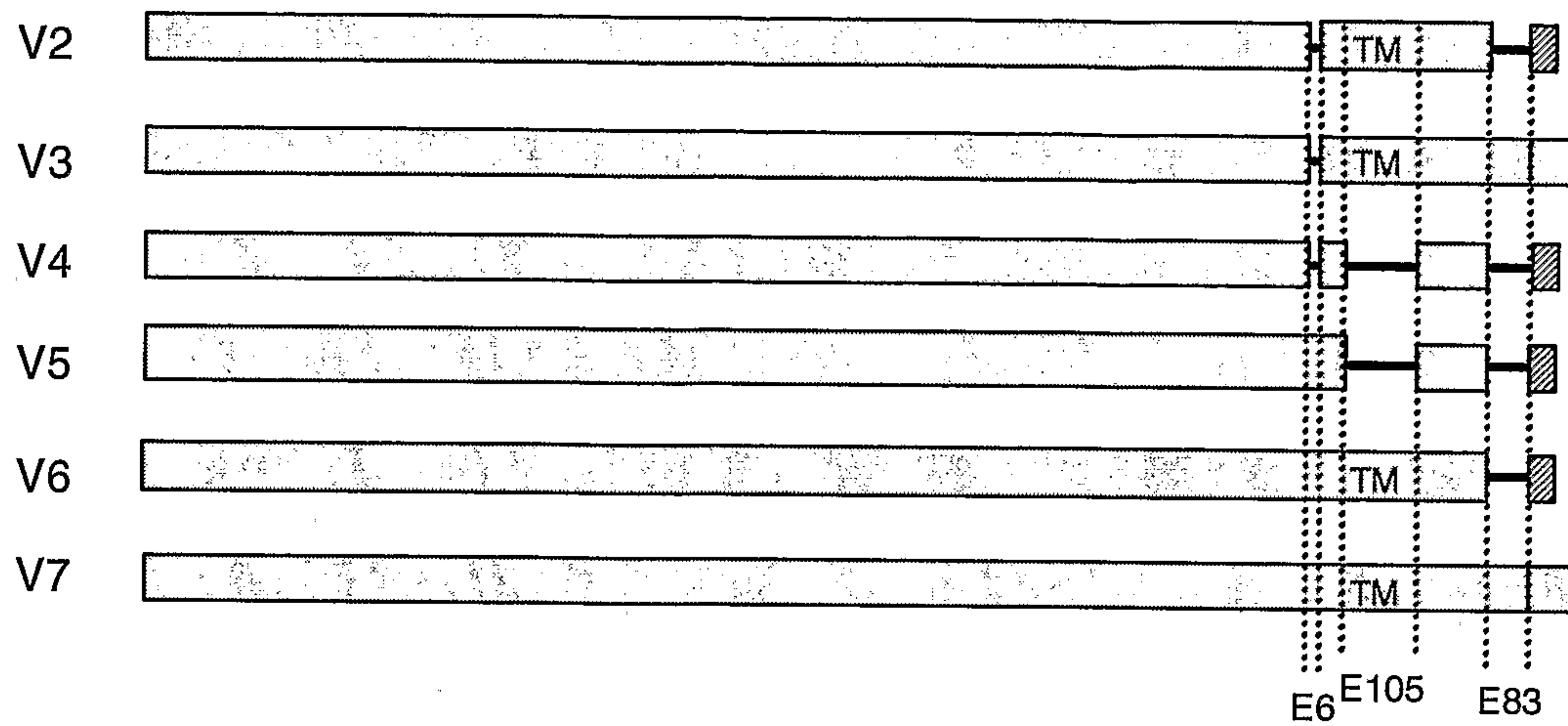


Figure 18

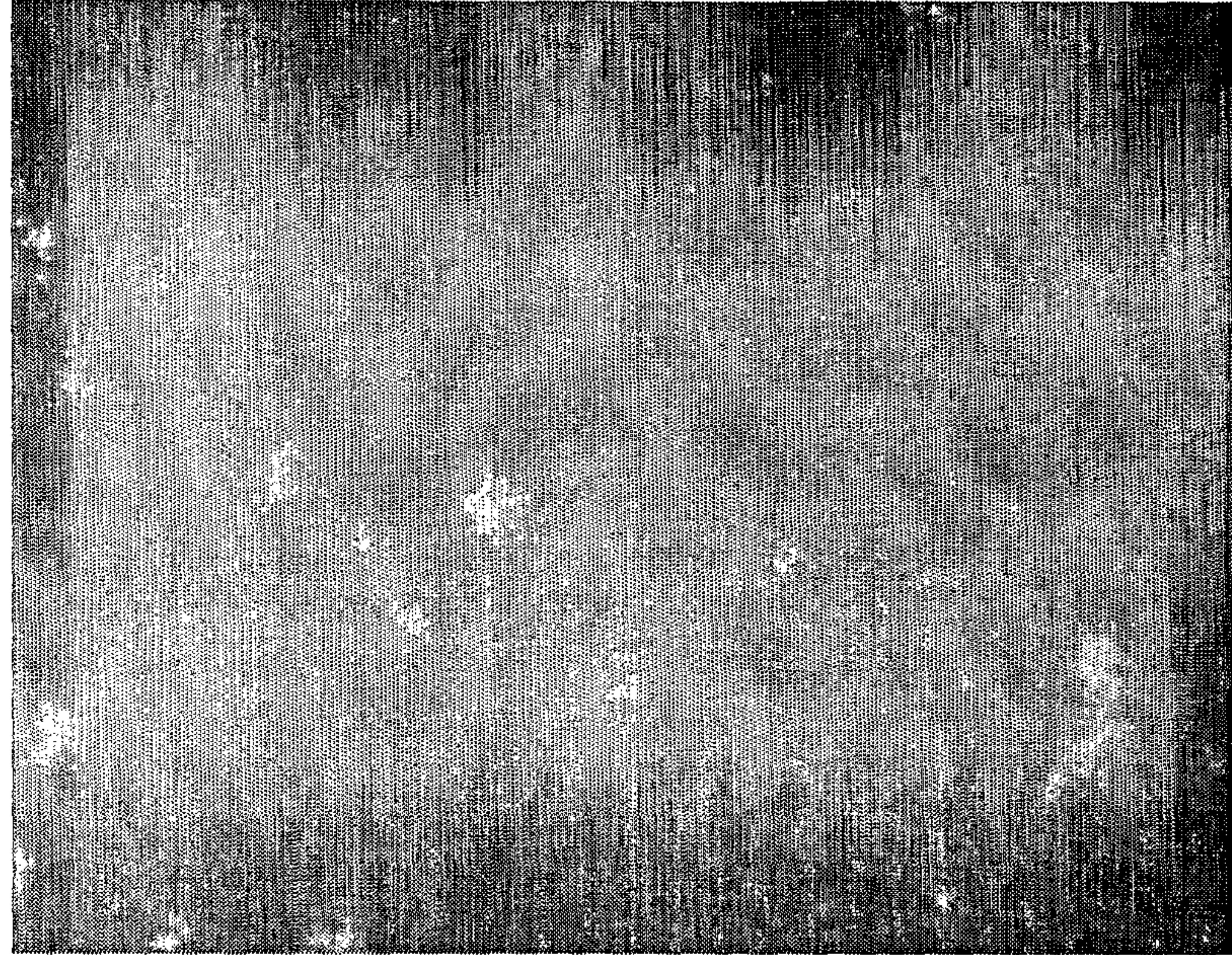


Figure 19

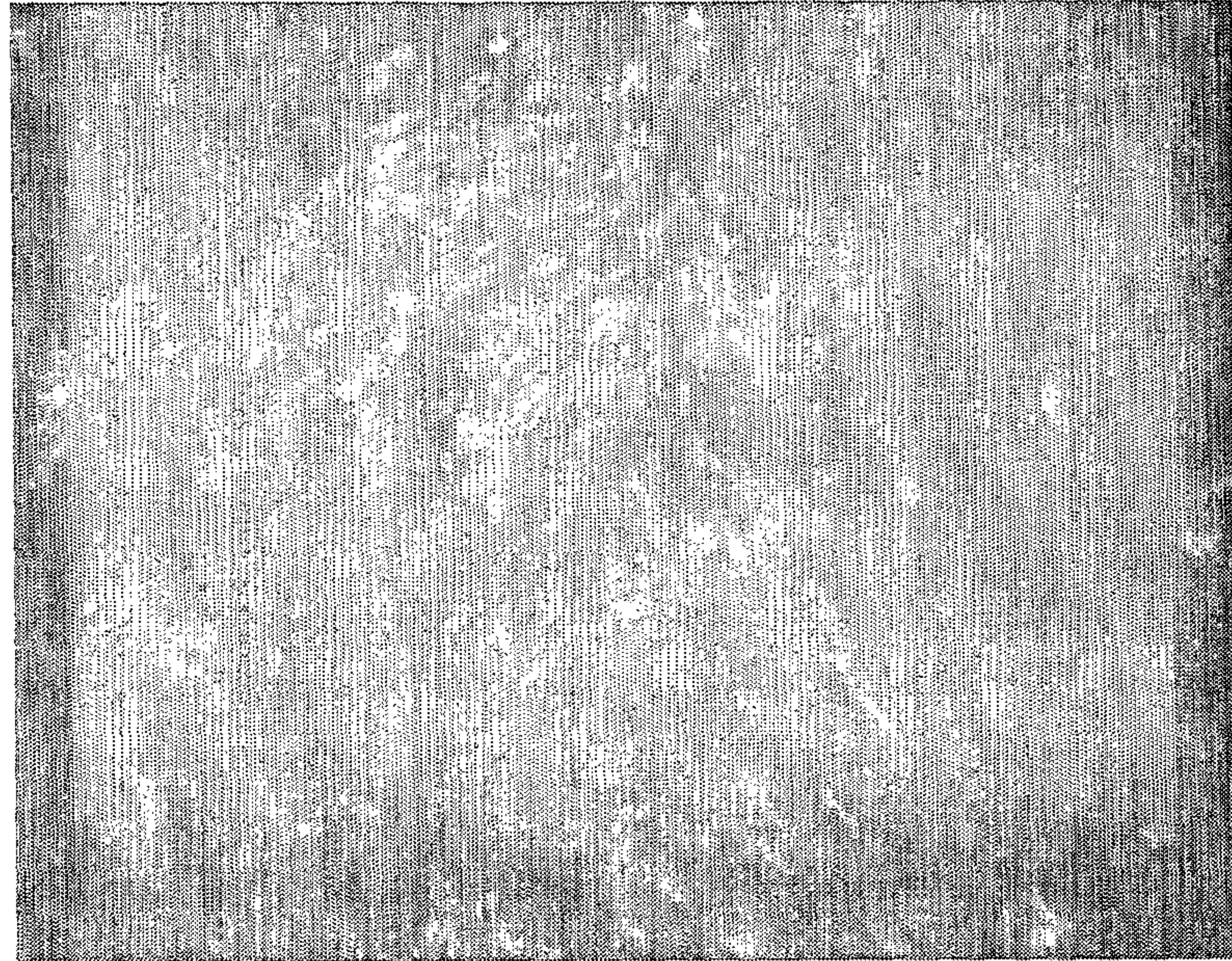


Figure 20A

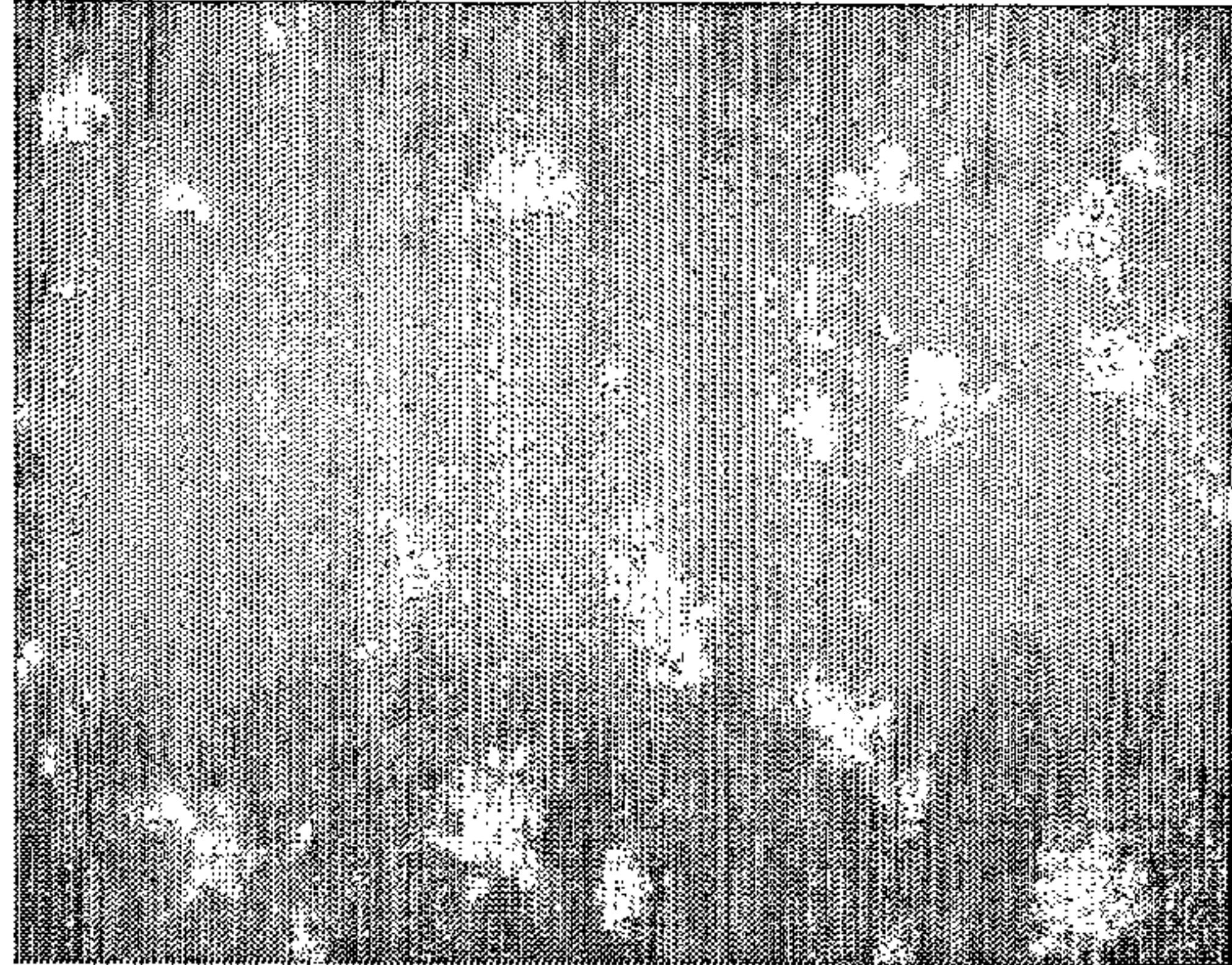


Figure 20B

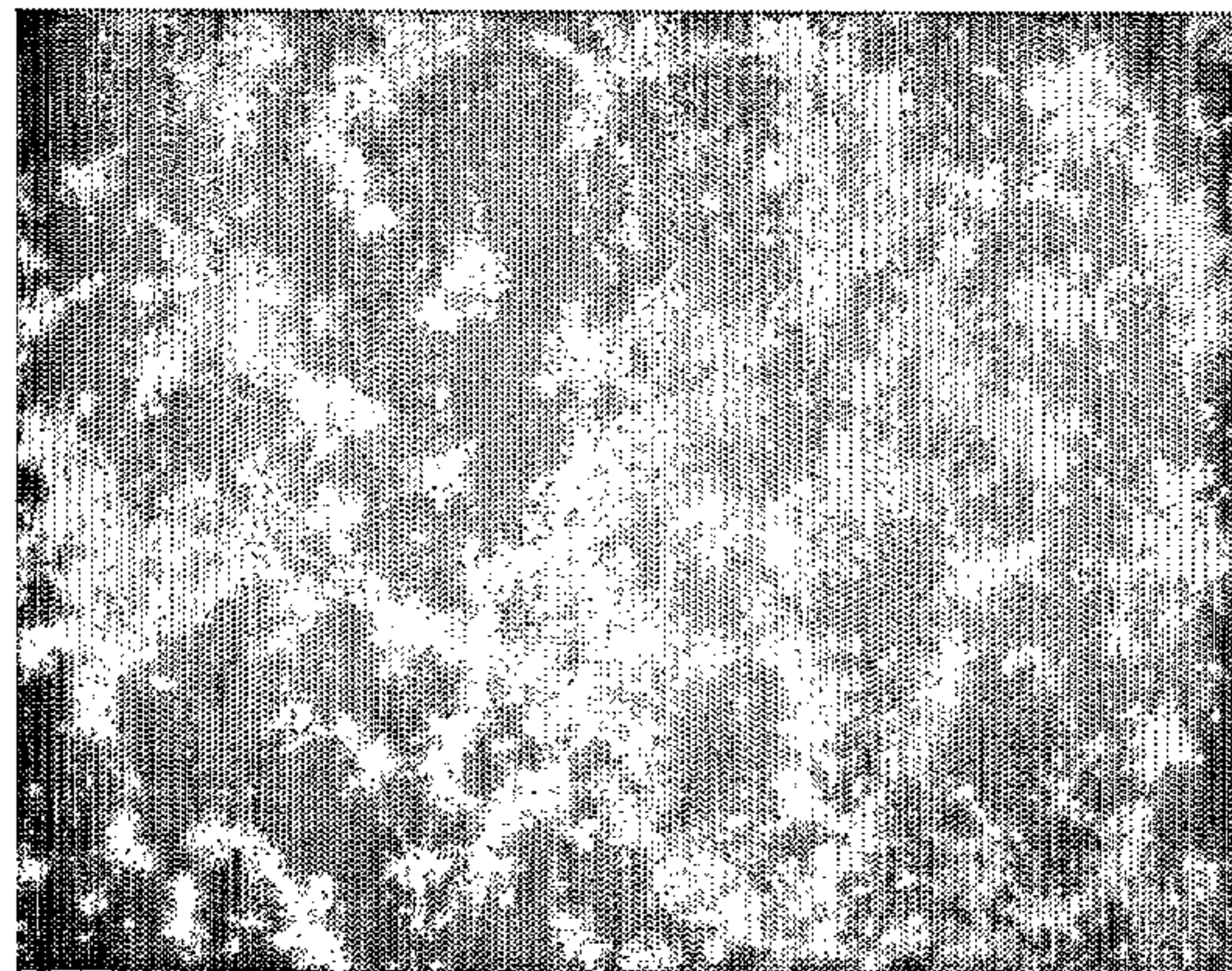


Figure 21A

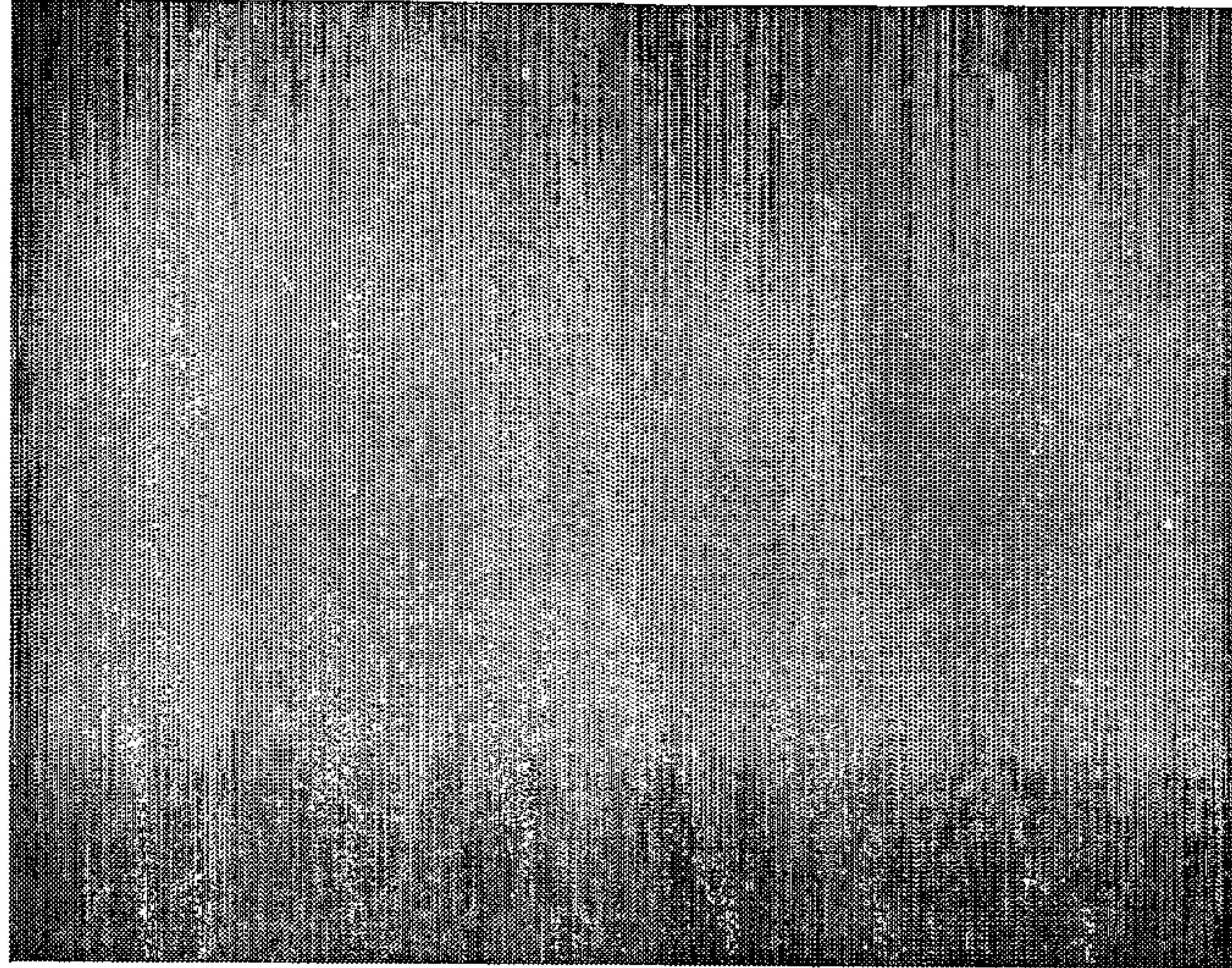


Figure 21B

