Exhibit C

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(54) **DNA ENCODING CANINE VON WILLEBRAND FACTOR AND METHODS OF USE**

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(58) **Field of Search** 536/22.1, 23.5, 536/24.31, 24.33; 435/325, 252.3, 6, 91.1, 91.2

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(57) **ABSTRACT**

The complete sequence of the canine von Willebrand Factor cDNA and deduced amino acid sequence is provided. The mutation which causes von Willebrand's Disease in Scottish Terriers, a single base deletion in exon 4, has also been determined. Methods for detecting carriers of the defective vWF gene are also provided.

15 Claims, 9 Drawing Sheets

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FIGURE 1A

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   1 CATTAANAGG TCCTGGCTGG GAGCTTTTTT TTGGGACCAG CACTCCATGT TCAAGGGCAA
  61 ACAGGGGCCA ATTAGGATCA ATCTTTTTTC TTTCTTTTTT TAAAAAAAAA AATTCTTCCC
 121 ACTTTGCACA CGGACAGTAG TACATACCAG TAGCTCTCTG CGAGGACGGT GATCACTAAT
 181 CATTTCTCCT GCTTCGTGGC AGATGAGTCC TACCAGACTT GTGAGGGTGC TGCTGGCTCT
 241 GGCCCTCATC TTGCCAGGGA AACTTTGTAC AAAAGGGACT GTTGGAAGGT CATCGATGGC
 301 CCGATGTAGC CTTCTCGGAG GTGACTTCAT CAACACCTTT GATGAGAGCA TGTACAGCTT
 361 TGCGGGAGAT TGCAGTTACC TCCTGGCTGG GGACTGCCAG GAACACTCCA TCTCACTTAT
 421 CGGGGGTTTC CAAAATGACA AAAGAGTGAG CCTCTCCGTG TATCTCGGAG AATTTTTCGA
 481 CATTCATTTG TTTGTCAATG GTACCATGCT GCAGGGGACC CAAAGCATCT CCATGCCCTA
 541 CGCCTCCAAT GGGCTGTATC TAGAGGCCGA GGCTGGCTAC TACAAGCTGT CCAGTGAGGC
 601 CTACGGCTTT GTGGCCAGAA TTGATGGCAA TGGCAACTTT CAAGTCCTGC TGTCAGACAG
 661 ATACTTCAAC AAGACCTGTG GGCTGTGTGG CAACTTTAAT ATCTTTGCTG AGGATGACTT
 721 CAAGACTCAA GAAGGGACGT TGACTTCGGA CCCCTATGAC TTTGCCAACT CCTGGGCCCT
 781 GAGCAGTGGG GAACAACGGT GCAAACGGGT GTCCCCTCCC AGCAGCCCAT GCAATGTCTC
 841 CTCTGATGAA GTGCAGCAGG TCCTGTGGGA GCAGTGCCAG CTCCTGAAGA GTGCCTCGGT
 901 GTTTGCCCGC TGCCACCCGC TGGTGGACCC TGAGCCTTTT GTCGCCCTGT GTGAAAGGAC
 961 TCTGTGCACC TGTGTCCAGG GGATGGAGTG CCCTTGTGCG GTCCTCCTGG AGTACGCCCG
1021 GGCCTGTGCC CAGCAGGGGA TTGTCTTGTA CGGCTGGACC GACCACAGCG TCTGCCGACC
1081 AGCATGCCCT GCTGGCATGG AGTACAAGGA GTGCGTGTCC CCTTGCACCA GAACTTGCCA
1141 GAGCCTTCAT GTCAAAGAAG TGTGTCAGGA GCAATGTGTA GATGGCTGCA GCTGCCCCGA
1201 GGGCCAGCTC CTGGATGAAG GCCACTGCGT GGGAAGTGCT GAGTGTTCCT GTGTGCATGC
1261 TGGGCAACGG TACCCTCCGG GCGCCTCCCT CTTACAGGAC TGCCACACCT GCATTTGCCG
1321 AAATAGCCTG TGGATCTGCA GCAATGAAGA ATGCCCAGGC GAGTGTCTGG TCACAGGACA
1381 GTCCCACTTC AAGAGCTTCG ACAACAGGTA CTTCACCTTC AGTGGGGTCT GCCACTACCT
1441 GCTGGCCCAG GACTGCCAGG ACCACACATT CTCTGTTGTC ATAGAGACTG TCCAGTGTGC
1501 CGATGACCTG GATGCTGTCT GCACCCGCTC GGTCACCGTC CGCCTGCCTG GACATCACAA
1561 CAGCCTTGTG AAGCTGAAGA ATGGGGGAGG AGTCTCCATG GATGGCCAGG ATATCCAGAT
1621 TCCTCTCCTG CAAGGTGACC TCCGCATCCA GCACACCGTG ATGGCCTCCG TGCGCCTCAG
1681 CTACGGGGAG GACCTGCAGA TGGATTCGGA CGTCCGGGGC AGGCTACTGG TGACGCTGTA
1741 CCCCGCCTAC GCGGGGAAGA CGTGCGGCCG TGGCGGGAAC TACAACGGCA ACGGGGGGGA
1801 CGACTTCGTG ACGCCCGCAG GCCTGGCGGA GCCCCTGGTG GAGGACTTCG GGAACGCCTG
1861 GAAGCTGCTC GGGGCCTGCG AGAACCTGCA GAAGCAGCAC CGCGATCCCT GCAGCCTCAA
1921 CCCGCGCCAG GCCAGGTTTG CGGAGGAGGC GTGCGCGCTG CTGACGTCCT CGAAGTTCGA
1981 GCCCTGCCAC CGAGCGGTGG GTCCTCAGCC CTACGTGCAG AACTGCCTCT ACGACGTCTG
2041 CTCCTGCTCC GACGGCAGAG ACTGTCTTTG CAGCGCCGTG GCCAACTACG CCGCAGCCGT
2101 GGCCCGGAGG GGCGTGCACA TCGCGTGGCG GGAGCCGGGC TTCTGTGCGC TGAGCTGCCC
2161 CCAGGGCCAG GTGTACCTGC AGTGTGGGAC CCCCTGCAAC ATGACCTGTC TCTCCCTCTC
2221 TTACCCGGAG GAGGACTGCA ATGAGGTCTG CTTGGAAAGC TGCTTCTCCC CCCCAGGGCT
2281 GTACCTGGAT GAGAGGGGAG ATTGTGTGCC CAAGGCTCAG TGTCCCTGTT ACTATGATGG
2341 TGAGATCTTT CAGCCCGAAG ACATCTTCTC AGACCATCAC ACCATGTGCT ACTGTGAGGA
2401 TGGCTTCATG CACTGTACCA CAAGTGGAGG CCTGGGAAGC CTGCTGCCCA ACCCGGTGCT
2461 CAGCAGCCCC CGGTGTCACC GCAGCAAAAG GAGCCTGTCC TGTCGGCCCC CCATGGTCAA
2521 GTTGGTGTGT CCCGCTGATA ACCCGAGGGC TGAAGGACTG GAGTGTGCCA AAACCTGCCA
2581 GAACTATGAC CTGCAGTGCA TGAGCACAGG CTGTGTCTCC GGCTGCCTCT GCCCGCAGGG
2641 CATGGTCCGG CATGAAAACA GGTGTGTGGC GCTGGAAAGA TGTCCCTGCT TCCACCAAGG
2701 CCAAGAGTAC GCCCCAGGAG AAACCGTGAA AATTGACTGC AACACTTGTG TCTGTCGGGA
2761 CCGGAAGTGG ACCTGCACAG ACCATGTGTG TGATGCCACT TGCTCTGCCA TCGGCATGGC
2821 GCACTACCTC ACCTTCGACG GACTCAAGTA CCTGTTCCCT GGGGAGTGCC AGTATGTTCT
2881 GGTGCAGGAT TACTGCGGCA GTAACCCTGG GACCTTACGG ATCCTGGTGG GGAACGAGGG
2941 GTGCAGCTAC CCCTCAGTGA AATGCAAGAA GCGGGTCACC ATCCTGGTGG AAGGAGGAGA
3001 GATTGAACTG TTTGATGGGG AGGTGAATGT GAAGAAACCC ATGAAGGATG AGACTCACTT
3061 TGAGGTGGTA GAGTCTGGTC AGTACGTCAT TCTGCTGCTG GGCAAGGCAC TCTCTGTGGT
3121 CTGGGACCAC CGCCTGAGCA TCTCTGTGAC CCTGAAGCGG ACATACCAGG AGCAGGTGTG
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FIGURE 1B

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3181 TGGCCTGTGT GGGAATTTTG ATGGCATCCA GAACAATGAT TTCACCAGCA GCAGCCTCCA
3241 AATAGAAGAA GACCCTGTGG ACTTTGGGAA TTCCTGGAAA GTGAACCCGC AGTGTGCCGA
3301 CACCAAGAAA GTACCACTGG ACTCATCCCC TGCCGTCTGC CACAACAACA TCATGAAGCA
3361 GACGATGGTG GATTCCTCCT GCAGGATCCT CACCAGTGAT ATTTTCCAGG ACTGCAACAG
3421 GCTGGTGGAC CCTGAGCCAT TCCTGGACAT TTGCATCTAC GACACTTGCT CCTGTGAGTC
3481 CATTGGGGAC TGCACCTGCT TCTGTGACAC CATTGCTGCT TACGCCCACG TCTGTGCCCA
3541 GCATGGCAAG GTGGTAGCCT GGAGGACAGC CACATTCTGT CCCCAGAATT GCGAGGAGCG
3601 GAATCTCCAC GAGAATGGGT ATGAGTGTGA GTGGCGCTAT AACAGCTGTG CCCCTGCCTG
3661 TCCCATCACG TGCCAGCACC CCGAGCCACT GGCATGCCCT GTACAGTGTG TTGAAGGTTG
3721 CCATGCGCAC TGCCCTCCAG GGAAAATCCT GGATGAGCTT TTGCAGACCT GCATCGACCC
3781 TGAAGACTGT CCTGTGTGTG AGGTGGCTGG TCGTCGCTTG GCCCCAGGAA AGAAAATCAT
3841 CTTGAACCCC AGTGACCCTG AGCACTGCCA AATTTGTAAT TGTGATGGTG TCAACTTCAC
3901 CTGTAAGGCC TGCAGAGAAC CCGGAAGTGT TGTGGTGCCC CCCACAGATG GCCCCATTGG
3961 CTCTACCACC TCGTATGTGG AGGACACGTC GGAGCCGCCC CTCCATGACT TCCACTGCAG
4021 CAGGCTTCTG GACCTGGTTT TCCTGCTGGA TGGCTCCTCC AAGCTGTCTG AGGACGAGTT
4081 TGAAGTGCTG AAGGTCTTTG TGGTGGGTAT GATGGAGCAT CTGCACATCT CCCAGAAGCG
4141 GATCCGCGTG GCTGTGGTGG AGTACCACGA CGGCTCCCAC GCCTACATCG AGCTCAAGGA
4201 CCGGAAGCGA CCCTCAGAGC TGCGGCGCAT CACCAGCCAG GTGAAGTACG CGGGCAGCGA
4261 GGTGGCCTCC ACCAGTGAGG TCTTAAAGTA CACGCTGTTC CAGATCTTTG GCAAGATCGA
4321 CCGCCCGGAA GCGTCTCGCA TTGCCCTGCT CCTGATGGCC AGCCAGGAGC CCTCAAGGCT
4381 GGCCCGGAAT TTGGTCCGCT ATGTGCAGGG CCTGAAGAAG AAGAAAGTCA TTGTCATCCC
4441 TGTGGGCATC GGGCCCCACG CCAGCCTTAA GCAGATCCAC CTCATAGAGA AGCAGGCCCC
4501 TGAGAACAAG GCCTTTGTGT TCAGTGGTGT GGATGAGTTG GAGCAGCGAA GGGATGAGAT
4561 TATCAACTAC CTCTGTGACC TTGCCCCCGA AGCACCTGCC CCTACTCAGC ACCCCCCAAT
4621 GGCCCAGGTC ACGGTGGGTT CGGAGCTGTT GGGGGTTTCA TCTCCAGGAC CCAAAAGGAA
4681 CTCCATGGTC CTGGATGTGG TGTTTGTCCT GGAAGGGTCA GACAAAATTG GTGAGGCCAA
4741 CTTTAACAAA AGCAGGGAGT TCATGGAGGA GGTGATTCAG CGGATGGACG TGGGCCAGGA
4801 CAGGATCCAC GTCACAGTGC TGCAGTACTC GTACATGGTG ACCGTGGAGT ACACCTTCAG
4861 CGAGGCGCAG TCCAAGGGCG AGGTCCTACA GCAGGTGCGG GATATCCGAT ACCGGGGTGG
4921 CAACAGGACC AACACTGGAC TGGCCCTGCA ATACCTGTCC GAACACAGCT TCTCGGTCAG
4981 CCAGGGGGAC CGGGAGCAGG TACCTAACCT GGTCTACATG GTCACAGGAA ACCCCGCTTC
5041 TGATGAGATC AAGCGGATGC CTGGAGACAT CCAGGTGGTG CCCATCGGGG TGGGTCCACA
5101 TGCCAATGTG CAGGAGCTGG AGAAGATTGG CTGGCCCAAT GCCCCCATCC TCATCCATGA
5161 CTTTGAGATG CTCCCTCGAG AGGCTCCTGA TCTGGTGCTA CAGAGGTGCT GCTCTGGAGA
5221 GGGGCTGCAG ATCCCCACCC TCTCCCCCAC CCCAGATTGC AGCCAGCCCC TGGATGTGGT
5281 CCTCCTCCTG GATGGCTCTT CCAGCATTCC AGCTTCTTAC TTTGATGAAA TGAAGAGCTT
5341 CACCAAGGCT TTTATTTCAA GAGCTAATAT AGGGCCCCGG CTCACTCAAG TGTCGGTGCT
5401 GCAATATGGA AGCATCACCA CTATCGATGT GCCTTGGAAT GTAGCCTATG AGAAAGTCCA
5461 TTTACTGAGC CTTGTGGACC TCATGCAGCA GGAGGGAGGC CCCAGCGAAA TTGGGGATGC
5521 TTTGAGCTTT GCCGTGCGAT ATGTCACCTC AGAAGTCCAT GGTGCCAGGC CCGGAGCCTC
5581 GAAAGCGGTG GTTATCCTAG TCACAGATGT CTCCGTGGAT TCAGTGGATG CTGCAGCCGA
5641 GGCCGCCAGA TCCAACCGAG TGACAGTGTT CCCCATTGGA ATCGGGGATC GGTACAGTGA
5701 GGCCCAGCTG AGCAGCTTGG CAGGCCCAAA GGCTGGCTCC AATATGGTAA GGCTCCAGCG
5761 AATTGAAGAC CTCCCCACCG TGGCCACCCT GGGAAATTCC TTCTTCCACA AGCTGTGCTC
5821 TGGGTTTGAT AGAGTTTGCG TGGATGAGGA TGGGAATGAG AAGAGGCCCG GGGATGTCTG
5881 GACCTTGCCA GACCAGTGCC ACACAGTGAC TTGCCTGCCA GATGGCCAGA CCTTGCTGAA
5941 GAGTCATCGG GTCAACTGTG ACCGGGGGCC AAGGCCTTCG TGCCCCAATG GCCAGCCCCC
6001 TCTCAGGGTA GAGGAGACCT GTGGCTGCCG CTGGACCTGT CCCTGTGTGT GCATGGGCAG
6061 CTCTACCCGG CACATCGTGA CCTTTGATGG GCAGAATTTC AAGCTGACTG GCAGCTGTTC
6121 GTATGTCCTA TTTCAAAACA AGGAGCAGGA CCTGGAGGTG ATTCTCCAGA ATGGTGCCTG
6181 CAGCCCTGGG GCGAAGGAGA CCTGCATGAA ATCCATTGAG GTGAAGCATG ACGGCCTCTC
6241 AGTTGAGCTC CACAGTGACA TGCAGATGAC AGTGAATGGG AGACTAGTCT CCATCCCATA
6301 TGTGGGTGGA GACATGGAAG TCAATGTTTA TGGGACCATC ATGTATGAGG TCAGATTCAA
6361 CCATCTTGGC CACATCTTCA CATTCACCCC CCAAAACAAT GAGTTCCAGC TGCAGCTCAG
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FIGURE 1C

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6421 CCCCAGGACC TTTGCTTCGA AGACATATGG TCTCTGTGGG ATCTGTGATG AGAACGGAGC
6481 CAATGACTTC ATTCTGAGGG ATGGGACAGT CACCACAGAC TGGAAGGCAC TCATCCAGGA
6541 ATGGACCGTA CAGCAGCTTG GGAAGACATC CCAGCCTGTC CATGAGGAGC AGTGTCCTGT
6601 CTCCGAATTC TTCCACTGCC AGGTCCTCCT CTCAGAATTG TTTGCCGAGT GCCACAAGGT
6661 CCTCGCTCCA GCCACCTTTT ATGCCATGTG CCAGCCCGAC AGTTGCCACC CGAAGAAAGT
6721 GTGTGAGGCG ATTGCCTTGT ATGCCCACCT CTGTCGGACC AAAGGGGTCT GTGTGGACTG
6781 GAGGAGGGCC AATTTCTGTG CTATGTCATG TCCACCATCC CTGGTGTACA ACCACTGTGA
6841 GCATGGCTGC CCTCGGCTCT GTGAAGGCAA TACAAGCTCC TGTGGGGACC AACCCTCGGA
6901 AGGCTGCTTC TGCCCCCCAA ACCAAGTCAT GCTGGAAGGT AGCTGTGTCC CCGAGGAGGC
6961 CTGTACCCAG TGCATCAGCG AGGATGGAGT CCGGCACCAG TTCCTGGAAA CCTGGGTCCC
7021 AGCCCACCAG CCTTGCCAGA TCTGCACGTG CCTCAGTGGG CGGAAGGTCA ACTGTACGTT
7081 GCAGCCCTGC CCCACAGCCA AAGCTCCCAC CTGTGGCCCG TGTGAAGTGG CCCGCCTCCG
7141 CCAGAACGCA GTGCAGTGCT GCCCGGAGTA CGAGTGTGTG TGTGACCTGG TGAGCTGTGA
7201 CCTGCCCCCG GTGCCTCCCT GCGAAGATGG CCTCCAGATG ACCCTGACCA ATCCTGGCGA
7261 GTGCAGACCC AACTTCACCT GTGCCTGCAG GAAGGATGAA TGCAGACGGG AGTCCCCGCC
7321 CTCTTGTCCC CCGCACCGGA CGCCGGCCCT TCGGAAGACT CAGTGCTGTG ATGAGTATGA
7381 GTGTGCATGC AACTGTGTCA ACTCCACGGT GAGCTGCCCG CTTGGGTACC TGGCCTCGGC
7441 TGTCACCAAC GACTGTGGCT GCACCACAAC AACCTGCTTC CCTGACAAGG TGTGTGTCCA
7501 CCGAGGCACC ATCTACCCTG TGGGCCAGTT CTGGGAGGAG GCCTGTGACG TGTGCACCTG
7561 CACGGACTTG GAGGACTCTG TGATGGGCCT GCGTGTGGCC CAGTGCTCCC AGAAGCCCTG
7621 TGAGGACAAC TGCCTGTCAG GCTTCACTTA TGTCCTTCAT GAAGGCGAGT GCTGTGGAAG
7681 GTGTCTGCCA TCTGCCTGTG AGGTGGTCAC TGGTTCACCA CGGGGCGACG CCCAGTCTCA
7741 CTGGAAGAAT GTTGGCTCTC ACTGGGCCTC CCCTGACAAC CCCTGCCTCA TCAATGAGTG
7801 TGTCCGAGTG AAGGAAGAGG TCTTTGTGCA ACAGAGGAAT GTCTCCTGCC CCCAGCTGAA
7861 TGTCCCCACC TGCCCCACGG GCTTCCAGCT GAGCTGTAAG ACCTCAGAGT GTTGTCCCAC
7921 CTGTCACTGC GAGCCCCTGG AGGCCTGCTT GCTCAATGGT ACCATCATTG GGCCGGGGAA
7981 AAGTCTGATG ATTGATGTGT GTACAACCTG CCGCTGCACC GTGCCGGTGG GAGTCATCTC
8041 TGGATTCAAG CTGGAGGGCA GGAAGACCAC CTGTGAGGCA TGCCCCCTGG GTTATAAGGA
8101 AGAGAAGAAC CAAGGTGAAT GCTGTGGGAG ATGTCTGCCT ATAGCTTGCA CCATTCAGCT
8161 AAGAGGAGGA CAGATCATGA CACTGAAGCG TGATGAGACT ATCCAGGATG GCTGTGACAG
8221 TCACTTCTGC AAGGTCAATG AAAGAGGAGA GTACATCTGG GAGAAGAGAG TCACGGGTTG
8281 CCCACCTTTC GATGAACACA AGTGTCTGGC TGAGGGAGGA AAAATCATGA AAATTCCAGG
8341 CACCTGCTGT GACACATGTG AGGAGCCAGA ATGCAAGGAT ATCATTGCCA AGCTGCAGCG
8401 TGTCAAAGTG GGAGACTGTA AGTCTGAAGA GGAAGTGGAC ATTCATTACT GTGAGGGTAA
8461 ATGTGCCAGC AAAGCCGTGT ACTCCATCCA CATGGAGGAT GTGCAGGACC AGTGCTCCTG
8521 CTGCTCGCCC ACCCAGACGG AGCCCATGCA GGTGGCCCTG CGCTGCACCA ATGGCTCCCT
8581 CATCTACCAT GAGATCCTCA ATGCCATCGA ATGCAGGTGT TCCCCCAGGA AGTGCAGCAA
8641 GTGAGGCCAC TGCCTGGATG CTACTGTCGC CTGCCTTACC CGACCTCACT GGACTGGCCA
8701 GAGTGCTGCT CAGTCCTCCT CAGTCCTCCT CCTGCTCTGC TCTTGTGCTT CCTGATCCCA
8761 CAATAAAGGT CAATCTTTCA CCTTGAAAAA AAAAAAAAAA AA
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Human   MIPARFAGVLLALALILPGTLCAEGTRGRSSTARCSLFGSDFVNTFDGSMYSFAGYCSYL     60
Dog     -S-T-LVR----------K--TK--V----M-----L-G--I----E-------D----

                                    *
Human   LAGGCQKRSFSIIGDFQNGKRVSLSVYLGEFFDIHLFVNGTVTQGDQRVSMPYASKGLYL    120
Dog     ---D--EH-I-L--G---D----------------------ML--T-SI------N----

Human   ETEAGYYKLSGEAYGFVARIDGSGNFQVLLSDRYFNKTCGLCGNFNIFAEDDFMTQEGTL    180
Dog     -A--------S----------N------------------------------K------

Human   TSDPYDFANSWALSSGEQWCERASPPSSSCNISSGEMQKGLWEQCQLLKSTSVFARCHPL    240
Dog     ------------------R-K-V-----P--V--D-V-QV----------A---------

Human   VDPEPFVALCEKTLCECAGGLECACPALLEYARTCAQEGMVLYGWTDHSACSPVCPAGME    300
Dog     ----------R---T-VQ-M--P-AV------A---Q-I---------V-R-A------

Human   YRQCVSPCARTCQSLHINEMCQERCVDGCSCPEGQLLDEGLCVESTECPCVHSGKRYPPG    360
Dog     -KE-----T-------VK-V---Q----------------H--G-A--S---A-Q-----

Human   TSLSRDCNTCICRNSQWICSNEECPGECLVTGQSHFKSFDNRYFTFSGICQYLLARDCQD    420
Dog     A--LQ--H-------L------------------------------V-H----Q----

Human   HSFSIVIETVQCADDRDAVCTRSVTVRLPGLHNSLVKLKHGAGVAMDGQDVQLPLLKGDL    480
Dog     -T--V----------L-------------H--------N-G--S-----I-I---Q---

Human   RIQHTVTASVRLSYGEDLQMDWDGRGRLLVKLSPVYAGKTCGLCGNYNGNQGDDFLTPSG    540
Dog     ------M-------------S-V------T-Y-A-------RG------R----V--A-

Human   LAEPRVEDFGNAWKLHGDCQDLQKQHSDPCALNPRMTRFSEEACAVLTSPTFEACHRAVS    600
Dog     ----L----------L-A-EN-----R---S----QA--A-----L---SK--P-----G

Human   PLPYLRNCRYDVCSCSDGRECLCGALASYAAACAGRGVRVAWREPGRCELNCPKGQVYLQ    660
Dog     -Q--VQ--L----------D---S-V-N----V-R---HI------F-A-S--Q------

Human   CGTPCNLTCRSLSYPDEECNEACLEGCFCPPGLYMDERGDCVPKAQCPCYYDGEIFQPED    720
Dog     ------M--L-----E-D---V---S--S-----L-[---]-------------------

Human   IFSDHHTMCYCEDGFMHCTMSGVPGSLLPDAVLSSPLSHRSKRSLSCRPPMVKLVCPADN    780
Dog     ------------------T--GL-----NP-----RC----------------------

Human   LRAEGLECTKTCQNYDLECMSMGCVSGCLCPPGMVRHENRCVALERCPCFHQGKEYAPGE    840
Dog     P-------A--------Q---T---------Q--------------------Q------

Human   TVKIGCNTCVCRDRKWNCTDHVCDATCSTIGMAHYLTFDGLKYLFPGECQYVLVQDYCGS    900
Dog     ----D-----------T----------A-------------------------------

Human   NPGTFRILVGNKGCSHPSVKCKKRVTILVEGGEIELFDGEVNVKRPMKDETHFEVVESGR    960
Dog     ----L------E---Y-------------------------K--------------Q

Human   YIILLLGKALSVVWDRHLSISVVLKQTYQEKVCGLCGNFDGIQNNDLTSSNLQVEEDPVD   1020
Dog     -V-------------HR-----T--R----Q---------------F---S--I------

Human   FGNSWKVSSQCADTRKVPLDSSPATCHNNIMKQTMVDSSCRILTSDVFQDCNKLVDPEPY   1080
Dog     -------NP-----K---------V--------------------I-----R------F
```

FIGURE 2A

```
Human   LDVCIYDTCSCESIGDCACFCDTIAAYAHVCAQHGKVVTWRTATLCPQSCEERNLRENGY  1140
Dog     --I--------------T--------------------A-----F---N------H----

Human   ECEWRYNSCAPACQVTCQHPEPLACPVQCVEGCHAHCPPGKILDELLQTCVDPEDCPVCE  1200
Dog     -------------PI-----------------------------------I---------

Human   VAGRRFASGKKVTLNPSDPEHCQICHCDVVNLTCEACQEPGGLVVPPTDAPVSPTTLYVE  1260
Dog     -----L-P---II------------N--G--F--K--R---SV------G-IGS--S---

Human   DISEPPLHDFYCSRLLDLVFLLDGSSRLSEAEFEVLKAFVVDMMERLRISQKWVRVAVVE  1320
Dog     -T--------H---------------K---D-------V---G---H-H----RI------

Human   YHDGSHAYIGLKDRKRPSELRRIASQVKYAGSQVASTSEVLKYTLFQIFSKIDRPEASRI  1380
Dog     ---------E-------------T--------E---------------G----------

Human   ALLLMASQEPQRMSRNFVRYVQGLKKKKVIVIPVGIGPHANLKQIRLIEKQAPENKAFVL  1440
Dog     ----------S-LA--L-----------------------S----H-------------F

Human   SSVDELEQQRDEIVSYLCDLAPEAPPPTLPPHMAQVTVGPGLLGVSTLGPKRNSMVLDVA  1500
Dog     -G------R----IN----------A--QH-P-------SE-----SP----------V

Human   FVLEGSDKIGEADFNRSKEFMEEVIQRMDVGQDSIHVTVLQYSYMVTVEYPFSEAQSKGD  1560
Dog     -----------N--K-R---------------R---------------T--------E

Human   ILQRVREIRYQGGNRTNTGLALRYLSDHSFLVSQGDREQAPNLVYMVTGNPASDEIKRLP  1620
Dog     V--Q--D---R----------Q---E---S--------V-----------------M-

Human   GDIQVVPIGVGPNANVQELERIGWPNAPILIQDFETLPREAPDLVLQRCCSGEGLQIPTL  1680
Dog     ------------H-------K----------H---M-----------------------

Human   SPAPDCSQPLDVILLLDGSSSFPASYFDEMKSFAKAFISKANIGPRLTQVSVLQYGSITT  1740
Dog     --T---------V--------I----------T-----R--------------------

Human   IDVPWNVVPEKAHLLSLVDVMQREGGPSQIGDALGFAVRYLTSEMHGARPGASKAVVILV  1800
Dog     -------AY--V-------L--Q-----E-----S-----V---V---------------

Human   TDVSVDSVDAAADAARSNRVTVFPIGIGDRYDAAQLRILAGPAGDSNVVKLQRIEDLPTM  1860
Dog     ------------E-----------------SE---SS----KAG--M-R---------V

Human   VTLGNSFLHKLCSGFVRICMDEDGNEKRPGDVWTLPDQCHTVTCQPDGQTLLKTHRVNCD  1920
Dog     A------F-------D-V-V-----------------------L--------S------

Human   RGLRPSCPNSQSPVKVEETCGCRWTCPCVCTGSSTRHIVTFDGQNFKLTGSCSYVLFQNK  1980
Dog     --P------G-P-LR--------------M-----------------------------

Human   EQDLEVILHNGACSPGARQGCMKSIEVKHSALSVELHSDMEVTVNGRLVSVPYVGGNMEV  2040
Dog     --------Q--------KET---------DG---------QM--------I-----D---

Human   NVYGAIMHEVRFNHLGHIFTFTPQNNEFQLQLSPKTFASKTYGLCGICDENGANDFMLRD  2100
Dog     ----T--Y-------------------------R--------------------I---

Human   GTVTTDWKTLVQEWTVQRPGQTCQPILEEQCLVPDSSHCQVLLLPLFAECHKVLAPATFY  2160
Dog     --------A-I------QL-K-S--VH----P-SEFF------SE--------------
```

FIGURE 2B

```
Human   AICQQDSCHQEQVCEVIASYAHLCRTNGVCVDWRTPDFCAMSCPPSLVYNHCEHGCPRHC   2220
Dog     -M--P----PKK---A--L-------K-------RAN--------------------L-

Human   DGNVSSCGDHPSEGCFCPPDKVMLEGSCVPEEACTQCIGEDGVQHQFLEAWVPDHQPCQI   2280
Dog     E--T-----Q---------NQ-----------------S----R-----T---A------

Human   CTCLSGRKVNCTTQPCPTAKAPTCGLCEVARLRQNADQCCPEYECVCDPVSCDLPPVPHC   2340
Dog     ------------L------------P----------V-----------L---------P-

Human   ERGLQPTLTNPGECRPNFTCACRKEECKRVSPPSCPPHRLPTLRKTQCCDEYECACNCVN   2400
Dog     -D---M------------------D--R-E---------T-A------------------

Human   STVSCPLGYLASTATNDCGCTTTTCLPDKVCVHRSTIYPVGQFWEEGCDVCTCTDMEDAV   2460
Dog     ------------AV-----------F--------G-----------A--------L--S-

Human   MGLRVAQCSQKPCEDSCRSGFTYVLHEGECCGRCLPSACEVVTGSPRGDSQSSWKSVGSQ   2520
Dog     ---------------N-L-----------------------------A--H--N---H

Human   WASPENPCLINECVRVKEEVFIQQRNVSCPQLEVPVCPSGFQLSCKTSACCPSCRCERME   2580
Dog     ----D----------------V----------N--T--T---------E---T-H--PL-

Human   ACMLNGTVIGPGKTVMIDVCTTCRCMVQVGVISGFKLECRKTTCNPCPLGYKEENNTGEC   2640
Dog     --L----I-----SL----------T-P----------G-----EA--------K-Q---

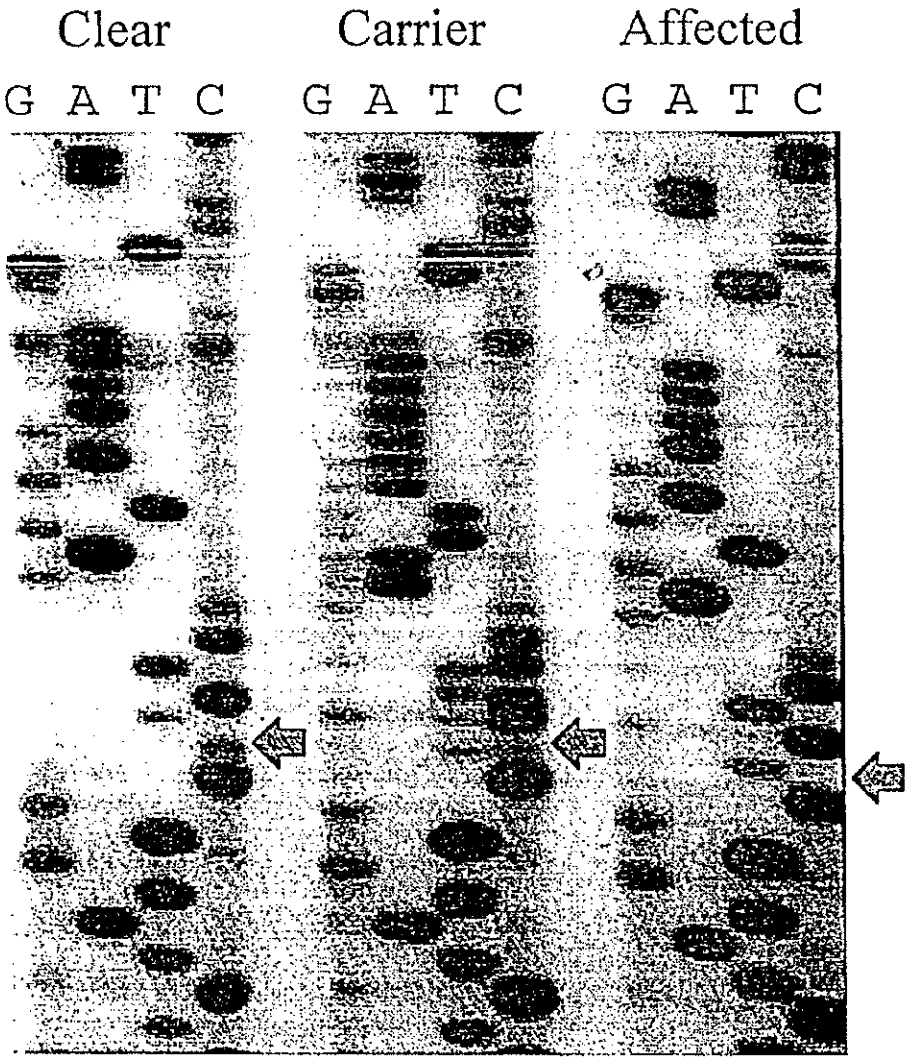
Human   CGRCLPTACTIQLRGGQIMTLKRDETLQDGCDTHFCKVNERGEYFWEKRVTGCPPFDEHK   2700
Dog     ------I------------------I-----S----------I---------------

Human   CLAEGGKIMKIPGTCCDTCEEPECNDITARLQYVKVGSCKSEVEVDIHYCQGKCASKAMY   2760
Dog     ------------------------K--I-K--R----D----E-------E-------V-

Human   SIDINDVQDQCSCCSPTRTEPMQVALHCTNGSVVYHEVLNAMECKCSPRKCSK         2813
Dog     --HME-----------Q--------R-----LI---I---I--R--------
```

FIGURE 2C

FIGURE 3



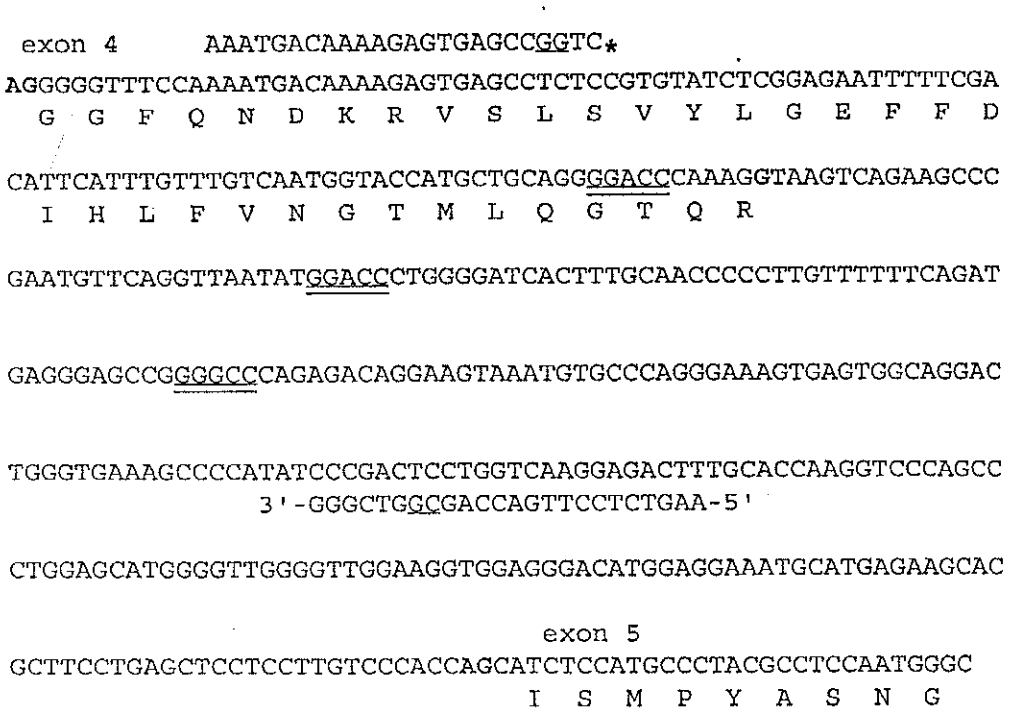
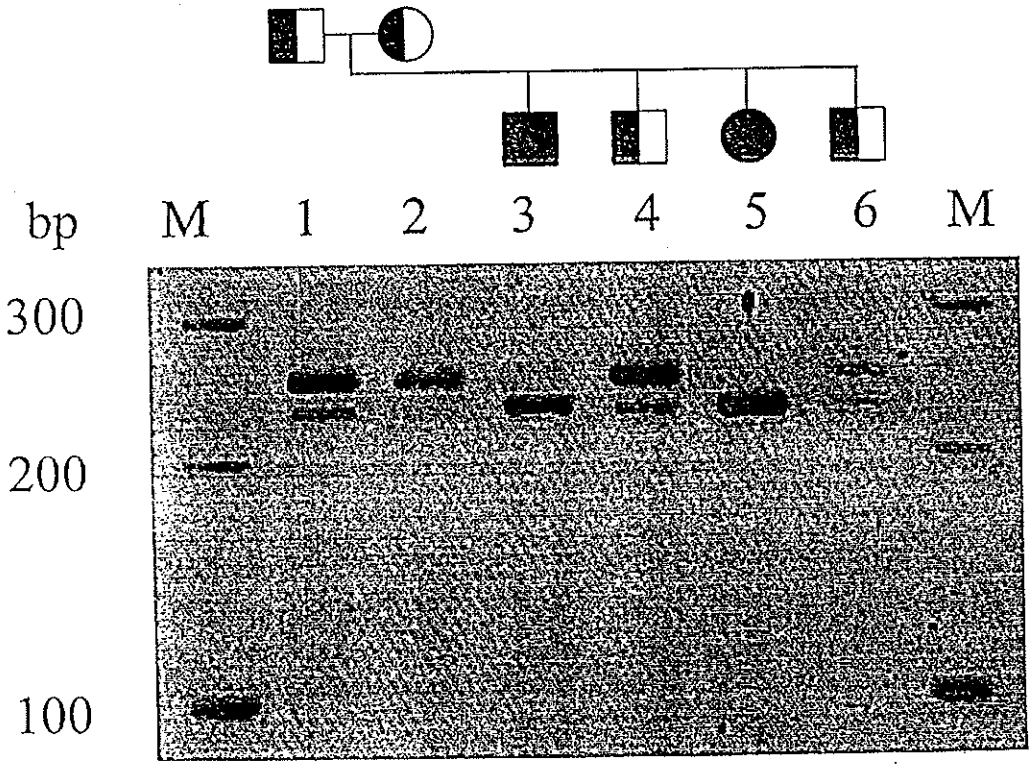

exon 4 AAATGACAAAAGAGTGAGCCGGTC*
AGGGGGTTTCCAAAATGACAAAAGAGTGAGCCTCTCCGTGTATCTCGGAGAATTTTTCGA
G G F Q N D K R V S L S V Y L G E F F D
CATTCATTTGTTTGTCAATGGTACCATGCTGCAGGGGACCCAAAGGTAAGTCAGAAGCCC
I H L F V N G T M L Q G T Q R
GAATGTTCAGGTTAATATGGACCCTGGGGATCACTTTGCAACCCCCTTGTTTTTTCAGAT
GAGGGAGCCGGGGCCCAGAGACAGGAAGTAAATGTGCCCAGGGAAAGTGAGTGGCAGGAC
TGGGTGAAAGCCCCATATCCCGACTCCTGGTCAAGGAGACTTTGCACCAAGGTCCCAGCC
3'-GGGCTGGCGACCAGTTCCTCTGAA-5'
CTGGAGCATGGGGTTGGGGTTGGAAGGTGGAGGGACATGGAGGAAATGCATGAGAAGCAC
exon 5
GCTTCCTGAGCTCCTCCTTGTCCCACCAGCATCTCCATGCCCTACGCCTCCAATGGGC
I S M P Y A S N G

FIGURE 4

FIGURE 5



DNA ENCODING CANINE VON WILLEBRAND FACTOR AND METHODS OF USE

This application is a continuation of Ser. No. 08/896,449, filed Jul. 18, 1997, now U.S. Pat. No. 6,040,143.

FIELD OF THE INVENTION

This invention relates generally to canine von willebrand factor (vWF), and more particularly, to the gene encoding vWF as well as a genetic defect that causes canine von Willebrand's disease.

BIOLOGICAL DEPOSITS

SEQUENCE	ACCESSION NO.
Canine von Willebrand Factor	AF 099154

BACKGROUND OF THE INVENTION

In both dogs and humans, von Willebrand's disease (vWD) is a bleeding disorder of variable severity that results from a quantitative or qualitative defect in von Willebrand factor (vWF) (Ginsburg, D. et al., *Blood* 79:2507–2519 (1992); Ruggeri, Z. M., et al., *FASEB J* 7:308–316 (1993); Dodds, W. J., *Mod Vet Pract* 681–686 (1984); Johnson, G. S. et al., *JAVMA* 176:1261–1263 (1988); Brooks, M., *Probl In Vet Med* 4:636–646 (1992)). This clotting factor has two known functions, stabilization of Factor VIII (hemophilic factor A) in the blood, and aiding the adhesion of platelets to the subendothelium, which allows them to provide hemostasis more effectively. If the factor is missing or defective, the patient, whether human or dog, may bleed severely.

The disease is the most common hereditary bleeding disorder in both species, and is genetically and clinically heterogenous. Three clinical types, called 1, 2, and 3 (formerly I, II, and III; see Sadler, J. E. et al., *Blood* 84:676–679 (1994) for nomenclature changes), have been described. Type 1 vWD is inherited in a dominant, incompletely penetrant fashion. Bleeding appears to be due to the reduced level of vWF rather than a qualitative difference. Although this is the most common form of vWD found in most mammals, and can cause serious bleeding problems, it is generally less severe than the other two types. In addition, a relatively inexpensive vasopressin analog (DDAVP) can help alleviate symptoms (Kraus, K. H. et al., *Vet Surg* 18:103–109 (1989)).

In Type 2 vWD, patients have essentially normal levels of vWF, but the factor is abnormal as determined by specialized tests (Ruggeri, Z. M., et al., *FASEB J* 7:308–316 (1993); Brooks, M., *Probl In Vet Med* 4:636–646 (1992)). This type is also inherited in a dominant fashion and has only rarely been described in dogs (Turrentine, M. A., et al., *Vet Clin North Am Small Anim Pract* 18:275 (1988)).

Type 3 vWD is the most severe form of the disease. It is inherited as an autosomal recessive trait, and affected individuals have no detectable vWF in their blood. Serious bleeding episodes require transfusions of blood or cryoprecipitate to supply the missing vWF. Heterozygous carriers have moderately reduced factor concentrations, but generally appear to have normal hemostasis.

Scottish terriers have Type 3 vWD (Dodds, W. J., *Mod Vet Pract* 681–686 (1984); Johnson, G. S. et al., *JAVMA* 176:1261–1263 (1988)). Homozygotes have no detectable vWF and have a severe bleeding disorder. Heterozygotes have reduced levels of the factor, and are clinically normal (Brooks, M. et al., *JAVMA* 200:1123–1127 (1992)). The prevalence of vWD among Scottish terriers including both heterozygotes and homozygotes has been variously estimated from 27–31% (Stokol, T. et al., *Res. Vet. Sci.* 59:152–155 (1995); Brooks, M., *Proc. 9th ACVIM Forum* 89–91 (1991)).

Currently, detection of affected and carrier Scottish terrier dogs is done by vWF antigen testing (Benson, R. E. et al., *Am J Vet Res* 44:399–403 (1983); Stokol, T. et al., *Res. Vet. Sci.* 59:152–155 (1995)) or by coagulation assays (Rosborough, T. K. et al., *J. Lab. Clin. Med.* 96:47–56 (1980); Read, M. S. et al., *J. Lab. Clin. Med.* 101:74–82 (1983)). These procedures yield variable results, as the protein-based tests can be influenced by such things as sample collection, sample handling, estrous, pregnancy, vaccination, age, and hypothyroidism (Strauss, H. S. et al., *New Eng J Med* 269:1251–1252 (1963); Bloom, A. L., *Mayo Clin Proc* 66:743–751 (1991); Stirling, Y. et al., *Thromb Haemostasis* 52:176–182 (1984); Mansell, P. D. et al., *Br. Vet. J.* 148:329–337 (1992); Avgeris, S. et al., *JAVMA* 196:921–924 (1990); Panciera, D. P. et al., *JAVMA* 205:1550–1553 (1994)). Thus, for example, a dog that tests within the normal range on one day, can test within the carrier range on another day. It is therefore difficult for breeders to use this information.

It would thus be desirable to provide the nucleic acid sequence encoding canine vWF. It would also be desirable to provide the genetic defect responsible for canine vWD. It would further be desirable to obtain the amino acid sequence of canine vWF. It would also be desirable to provide a method for detecting carriers of the defective vWF gene based on the nucleic acid sequence of the normal and defective vWF gene.

SUMMARY OF THE INVENTION

The present invention provides a novel purified and isolated nucleic acid sequence encoding canine vWF. A nucleic acid sequence containing the mutation that causes vWD in Scottish terriers, a single-base deletion in exon 4, is also provided. The nucleic acid sequences of the present invention may be used in methods for detecting carriers of the mutation that causes vWD. Such methods may be used by breeders to reduce the frequency of the disease-causing allele and the incidence of disease. In addition, the nucleic acid sequence of the canine vWF provided herein may be used to determine the genetic defect that causes vWD in other breeds as well as other species.

Additional objects, advantages, and features of the present invention will become apparent from the following description, taken in conjunction with the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

The various advantages of the present invention will become apparent to one skilled in the art by reading the following specification and by referencing the following drawings in which:

FIGS. 1A–1C is the nucleic acid sequence of the canine von Willebrand factor of the present invention;

FIGS. 2A–2C is a comparison of the human and canine prepro-von Willebrand factor amino acid sequences;

FIG. 3 provides nucleotide sequencing ladders for the von Wilebrand's disease mutation region for normal (clear), carrier, and affected Scottish terriers, the sequences being obtained directly from PCR products derived from genomic DNAs in exon 4;

FIG. 4 illustrates the results of a method of the present invention used to detect the Scottish terrier vWD mutation; and

FIG. 5 shows the Scottish terrier pedigree, which in turn illustrates segregation of the mutant and normal vWF alleles.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The cDNA encoding canine von Willebrand Factor (vWF) has been sequenced, and its sequence is set forth in FIGS. 1A–1C and SEQ ID NO: 1. The amino acid sequence corresponding to the cDNA of canine vWF has been subsequently deduced and is set forth in FIGS. 2A–2C and SEQ ID NO: 2. The mutation of the normal vWF gene which causes von Willebrand's Disease (vWD), a deletion at codon 88 of the normal gene resulting in a frameshift, is also provided. The nucleic acid sequences of the present invention may be used in methods for detecting homozygous and heterozygous carriers of the defective vWF gene.

In a preferred method of detecting the presence of the von Willebrand allele in canines, DNA samples are first collected by relatively noninvasive techniques, i.e., DNA samples are obtained with minimal penetration into body tissues of the animals to be tested. Common noninvasive tissue sample collection methods may be used and include withdrawing buccal cells via cheek swabs and withdrawing blood samples. Following isolation of the DNA by standard techniques, PCR is performed on the DNA utilizing predesigned primers that produce enzyme restriction sites on those DNA samples that harbor the defective gene. Treatment of the amplified DNA with appropriate restriction enzymes such as BsiE I thus allows one to analyze for the presence of the defective allele. One skilled in the art will appreciate that this method may be applied not only to Scottish terriers, but to other breeds such as Shetland sheepdogs and Dutch Kooikers.

Overall, the present invention provides breeders with an accurate, definitive test whereby the undesired vWD gene may be eliminated from breeding lines. The current tests used by breeders are protein- based, and as noted previously, the primary difficulty with this type of test is the variability of results due to a variety of factors. The ultimate result of such variability is that an inordinate number of animals fall into an ambiguous grouping whereby carriers and noncarriers cannot be reliably distinguished. The present invention obviates the inherent limitations of protein-based tests by detecting the genetic mutation which causes vWD. As described in Specific Example 1, the methods of the present invention provide an accurate test for distinguishing noncarriers, homozygous carriers and heterozygous carriers of the defective vWF gene.

It will be appreciated that because the vWF cDNA of the present invention is substantially homologous to vWF cDNA throughout the canine species, the nucleic acid sequences of the present invention may be used to detect DNA mutations in other breeds as well. In addition, the canine vWF sequence presented herein potentially in combination with the established human sequence (Genbank Accession No. X04385, Bonthron, D. et al., *Nucleic Acids Res.* 14:7125–7128 (1986); Mancuso, D. J. et al., *Biochemistry* 30:253–269 (1989); Meyer, D. et al., *Throm Haemostasis* 70:99–104 (1993)), may be used to facilitate sequencing of the vWF gene and genetic defects causing vWD, in other mammalian species e.g., by using cross-species PCR methods known by those skilled in the art.

It is also within the contemplation of this invention that the isolated and purified nucleic acid sequences of the present invention be incorporated into an appropriate recombinant expression vector, e.g., viral or plasmid, which is capable of transforming an appropriate host cell, either eukaryotic (e.g., mammalian) or prokaryotic (e.g., *E. coli*). Such DNA may involve alternate nucleic acid forms, such as cDNA, gDNA, and DNA prepared by partial or total chemical synthesis. The DNA may also be accompanied by additional regulatory elements, such as promoters, operators and regulators, which are necessary and/or may enhance the expression of the vWF gene product. In this way, cells may be induced to over-express the vWF gene, thereby generating desired amounts of the target vWF protein. It is further contemplated that the canine vWF polypeptide sequence of the present invention may be utilized to manufacture canine vWF using standard synthetic methods. One skilled in the art will also note that the defective protein encoded by the defective vWF gene of the present invention may also be of use in formulating a complementary diagnostic test for canine vWD that may provide further data in establishing the presence of the defective allele. Thus, production of the defective vWF polypeptide, either through expression in transformed host cells as described above for the active vWF polypeptide or through chemical synthesis, is also contemplated by the present invention.

The term "gene" as to referred herein means a nucleic acid which encodes a protein product. The term "nucleic acid" refers to a linear array of nucleotides and nucleosides, such as genomic DNA, cDNA and DNA prepared by partial or total chemical synthesis from nucleotides. The term "encoding" means that the nucleic acid may be transcribed and translated into the desired polypeptide. "Polypeptide" refers to amino acid sequences which comprise both full-length proteins and fragments thereof. "Mutation" as referred to

herein includes any alteration in a nucleic acid sequence including, but not limited to, deletions, substitutions and additions.

As referred to herein, the term "capable of hybridizing under high stringency conditions" means annealing a strand of DNA complementary to the DNA of interest under highly stringent conditions. Likewise, "capable of hybridizing under low stringency conditions" refers to annealing a strand of DNA complementary to the DNA of interest under low stringency conditions. In the present invention, hybridizing under either high or low stringency conditions would involve hybridizing a nucleic acid sequence (e.g., the complementary sequence to SEQ ID NO: 1 or portion thereof), with a second target nucleic acid sequence. "High stringency conditions" for the annealing process may involve, for example, high temperature and/or low salt content, which disfavor hydrogen bonding contacts among mismatched base pairs. "Low stringency conditions" would involve lower temperature, and/or lower salt concentration than that of high stringency conditions. Such conditions allow for two DNA strands to anneal if substantial, though not near complete complementarity exists between the two strands, as is the case among DNA strands that code for the same protein but differ in sequence due to the degeneracy of the genetic code. Appropriate stringency conditions which promote DNA hybridization, for example, 6×SSC at about 45° C., followed by a wash of 2×SSC at 50° C. are known to those skilled in the art or can be found in *Current Protocols in Molecular Biology*, John Wiley & Sons, NY (1989), 6.31–6.3.6. For example, the salt concentration in the wash step can be selected from a low stringency of about 2×SSC at 50° C. to a high stringency of about 0.2×SSC at 50° C. In addition, the temperature in the wash step can be increased from low stringency at room temperature, about 22° C., to high stringency conditions, at about 65° C. Other stringency parameters are described in Maniatis, T., et al., *Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press*, Cold Spring N.Y., (1982), at pp. 387–389; see also Sambrook J. et al., *Molecular Cloning: A Laboratory Manual*, Second Edition, Volume 2, Cold Spring Harbor Laboratory Press, Cold Spring, N.Y. at pp. 8.46–8.47 (1989).

SPECIFIC EXAMPLE 1

Materials And Methods

Isolation of RNA. The source of the RNA was a uterus from a Scottish Terrier affected with vWD (factor level <0.1% and a clinical bleeder), that was surgically removed because of infection. Spleen tissue was obtained from a Doberman Pinscher affected with vWD that died from dilated cardiomyopathy (factor level 7% and a clinical bleeder). Total RNA was extracted from the tissues using Trizol (Life Technologies, Gaithersburg, Md.). The integrity of the RNA was assessed by agarose gel electrophoresis.

Design of PCR primer sets. Primers were designed to a few regions of the gene, where sequences from two species were available (Lavergne, J. M. et al., *Biochem Biophys Res Commun* 194:1019–1024 (1993); Bakhshi, M. R. et al., *Biochem Biophys Acta* 1132:325–328 (1992)). These primers were designed using rules for cross-species' amplifications (Venta et al., "Genes-Specific Universal Mammalian Sequence-Tagged Sites: Application To The Canine Genome" *Biochem. Genet.* (1996) in press). Most of the primers had to be designed to other regions of the gene using the human sequence alone (Mancuso, D. J. et al., *Biochemistry* 30:253–269 (1991)). Good amplification conditions were determined by using human and canine genomic DNAs.

Reverse Transcriptase-PCR. Total RNA was reverse transcribed using random primers (Bergenhem, N. C. H. et al., *PNAS* (USA) 89:8789–8802 (1992)). The cDNA was amplified using the primer sets shown to work on canine genomic DNA.

DNA Sequence Analysis. Amplification products of the predicted sizes were isolated from agarose gels by adsorption onto silica gel particles using the manufacturer's method (Qiagen, Chatsworth, Calif.). Sequences were determined using ^{32}P-5' end-labeled primers and a cycle sequencing kit (United States Biochemical Corp., Cleveland, Ohio). The sequences of the 5' and 3' untranslated regions were determined after amplification using Marathon™ RACE kits (Clontech, Palo Alto, Calif.). Sequences were aligned using the Eugene software analysis package (Lark Technologies, Houston, Tex.). The sequence of the canine intron four was determined from PCR-amplified genomic DNA.

Design of a Diagnostic Test. PCR mutagenesis was used to create diagnostic and control BsiE I and Sau96 I restriction enzyme sites for the test. Amplification conditions for the test are: 94° C., 1 min, 61° C., 1 min, and 72° C., 1 min, for 50 cycles using cheek swab DNA (Richards, B. et al., *Human Molecular Genetics* 2:159–163 (1992)).

Population Survey. DNA was collected from 87 Scottish terriers from 16 pedigrees. DNA was isolated either from blood using standard procedures (Sambrook, J. et al., Cold Harbor Spring Lab, Cold Harbor Spring N.Y., 2nd Edition, (1989)) or by cheek swab samples (Richards, B. et al., *Human Molecular Genetics* 2:159–163 (1992)). The genetic status of each animal in the survey was determined using the BsiE I test described above.

Results

Comparison of the canine and human sequences. The alignment of the canine and human prepro-von Willebrand Factor amino acid sequences is shown in FIGS. 2A–2C. The location of the Scottish terrier vWD mutation is indicated by the "*". Potential N-glycosylation sites are shown in bold type. The known and postulated integrin binding sites are boxed. Amino acid numbers are shown on the right side of the figure. The human sequence is derived from Genbank accession number X04385 (Bonthron, D. et al., *Nucleic Acids Res.* 14:7125–7128 (1986)).

Overall, 85.1% sequence identity is seen between the prepro-vWF sequences. The pro-region is slightly less conserved than the mature protein (81.4% vs. 87.5%). There were no other noteworthy percentage sequence identity

differences seen in other regions of the gene, or between the known repeats contained within the gene (data not shown). Fourteen potential N-linked glycosylation sites are present in the canine sequence, all of which correspond to similar sites contained within the human sequence. The two integrin binding sites identified in the human vWF protein sequence (Lankhof, H. et al., *Blood* 86:1035–1042 (1995)) are conserved in the canine sequence as well (FIGS. 2A–2C). The 5' and 3' untranslated regions have diverged to a greater extent than the coding region (data not shown), comparable to that found between the human and bovine sequences derived for the 5' flanking region (Janel, N. et al., *Gene* 167:291–295 (1995)). Additional insights into the structure and function of the von Willebrand factor can be gained by comparison of the complete human sequence (Mancuso, D. J. et al., *Biochemistry* 30:253–269 (1989); Meyer, D. et al., *Throm Haemostasis* 70:99–104 (1993)) and the complete canine sequence reported here.

The sequence for most of exon 28 was determined (Mancuso, D. J. et al., *Thromb Haemost* 69:980 (1993); Porter, C. A. et al., *Mol Phylogenet Evol* 5:89–101 (1996)). All three sequences are in complete agreement, although two silent variants have been found in other breeds (Table 1, exon 28). Partial sequences of exons 40 and 41 (cDNA nucleotide numbers 6923 to 7155, from the initiation codon) were also determined as part of the development of a polymorphic simple tandem repeat genetic marker (Shibuya, H. et al., *Anim Genet* 24:122 (1994)). There is a single nucleotide sequence difference between this sequence ("T") and the sequence of the present invention, ("C") at nucleotide position 6928.

Scottish Terrier vWD mutation. FIG. **3** shows nucleotide sequencing ladders for the von Willebrand's Disease mutation region for normal (clear), carrier, and affected Scottish terriers. The sequences were obtained directly from PCR products derived from genomic DNAs in exon 4. The arrowheads show the location of the C nucleotide that is deleted in the disease-causing allele. Note that in the carrier ladder each base above the point of the mutation has a doublet appearance, as predicted for deletion mutations. The factor levels reported for these animals were: Normal, 54%; Carrier, 34%; Affected, <0.1%.

As a result of the deletion, a frameshift mutation at codon 88 leads to a new stop codon 103 bases downstream. The resulting severely truncated protein of 119 amino acids does not include any of the mature von Willebrand factor region. The identity of the base in the normal allele was determined from an unaffected dog.

Development of a diagnostic test. A PCR primer was designed to produce a BsiE I site in the mutant allele but not in the normal allele (FIG. **4**). The position of the deleted nucleotide is indicated by an asterisk. The altered nucleotides in each primer are underlined. The normal and mutant allele can also be distinguished using Sau96 I. The naturally occurring Sau96 I sites are shown by double underlines. The highly conserved donor and acceptor dinucleotide splice sequences are shown in bold type.

In order to ensure that the restriction enzyme cut the amplified DNA to completion, an internal control restriction site common to both alleles was designed into the non-diagnostic primer. The test was verified by digestion of the DNA from animals that were affected, obligate carriers, or normal (based on high factor levels [greater than 100% of normal] obtained from commonly used testing labs and reported to us by the owners, and also using breeds in which Type 3 vWD has not been observed). The expected results were obtained (e.g., FIG. **5**). Five vWD-affected animals from a colony founded from Scottish terriers (Brinkhous, K. M. et al., *Ann. New York Acad. Sci.* 370:191–203 (1981)) were also shown to be homozygous for this mutation. An additional unaffected animal from this same colony was found to be clear.

It would still be possible to misinterpret the results of the test if restriction enzyme digestion was not complete, and if the rates of cleavage of the cont778rol and diagnostic sites were vastly different. The rates of cleavage of the two BsiE I sites were thus examined by partially digesting the PCR products and running them on capillary electrophoresis. The rates were found to be very nearly equal (the diagnostic site is cut 12% faster than the control site).

The mutagenesis primer was also designed to produce a Sau96 I site into the normal allele but not the mutant allele. This is the reverse relationship compared to the BsiE I-dependent test, with respect to which allele is cut. Natural internal Sau96 I sites serve as digestion control sites (shown in FIG. **4**). The test using this enzyme produced identical genotypic results compared to the BsiE I for all animals examined (data not shown).

A possible mutation in the Doberman Pinscher gene. The complete Scottish terrier sequence was compared to the complete Doberman Pinscher sequence. Several nucleotide differences were found and were compared to the nucleotides found in the same position in the human sequence as shown in Table 1 below. Most of these changes were silent. However, of three amino acid changes, one is relatively non-conservative (F905L) and is proposed to be the mutation that causes Doberman Pinscher vWD. Other data strongly suggest that the nucleotide interchange at the end of exon 43 causes a cryptic splice site to be activated reducing the amount of normally processed mRNA, with a concomitant decrease in the amount of vWF produced.

Mendelian inheritance. One test often used to verify the correct identification of a mutant allele is its inheritance according to Mendel's law of segregation. Three pedigrees were examined in which the normal and mutant alleles were segregating, as shown in FIG. **5**. Exon four of the vWF gene was PCR-amplified from genomic DNA. The PCR products were examined for the presence of the normal and mutant vWF alleles by agarose gel electrophoresis after digestion with BsiE I (see FIG. **5**). The affected animals are homozygous for the mutant allele (229 bp; lanes 3 and 5). The other animals in this pedigree are heterozygotes (251 bp and 229 bp; lanes 1, 2, 4, and 6), including the obligate carrier parents.

TABLE 1

Differences Between Scottie And Doberman
Protein And Nucleotide von Willebrand Factor Sequences
With Comparison To The Human Sequences

		Amino Acid			Codon		
Exon	A.A.[1]	Human	Scottie	Doberman	Human	Scottie	Doberman
5' UT[2]	nuc-35[3]	N/A[4]	N/A	N/A	N/A	A	G
4	85	S	S/F.Shift[5]	S	TCC	TTC/TC_	TCC
5	173	M	R	K	ATG	AGG	AAG
11	422	S	T	T	TCC	ACA	ACC
21	898	C	C	C	TGC	TGT	TGC
21	905	F	F	L	TTT	TTC	TTA
24	1041	S	S	S	TCA	TCA	TCG
24	1042	S	S	S	TCC	TCC	TCA
28	1333	D	D	E	GAC	GAC	GAG
28	1349	Y	Y	Y	TAT	TAT	TAC*
42	2381	P	L	P	CCC	CTG	CCG
43	2479	S	S	S	TCG	TCG	TCA
45	2555	P	P	P	CCC	CCC	CCG
47	2591	P	P	P	CCC	CCT	CCC
49	2672	D	D	D	GAT	GAT	GAC
51	2744	E	E	E	GAG	GAG	GAA

[1]Amino acid residue position
[2]Untranslated region
[3]Nucleotide position
[4]Not Applicable
[5]Frameshift mutation
Boxed residues show amino acid differences between breeds
*This site has been shown to be polymorphic in some breeds
The mature VWF protein begins in exon 18

The alleles, as typed by both the BsiE I and Sau96 I tests, showed no inconsistencies with Mendelian inheritance. One of these pedigrees included two affected animals, two phenotypically normal siblings, and the obligate carrier parents. The two parents were found to be heterozygous by the test, the two affected animals were found to be homozygous for the mutant allele, and the normal siblings were found to be heterozygotes.

Population survey for the mutation. Cheek swabs or blood samples were collected from 87 animals in order to determine the incidence of carriers in the U.S. Scottish terrier population. Although we attempted to make the sample as random as possible, these dogs were found to come from 16 pedigrees, several of which are more distantly interconnected. This is due to some ascertainment bias, based on ownership (as opposed to phenotypic ascertainment bias). In these 87 animals four affected and 15 carrier animals were found.

Discussion

These results establish that the single base deletion found in exon four of the vWF gene causes vWD in the Scottish terrier breed. The protein produced from the mutant allele is extremely short and does not include any of the mature vWF protein. Four Scottish terriers known to be affected with the disease are homozygous for the mutation. Five other mixed-breed dogs descended from Scottish terriers, and affected with vWD, are also homozygous for the mutation. No normal animals are homozygous for the mutation. Unaffected obligate carriers are always heterozygous for the mutation.

The gene frequency, as determined from the population survey, appears to be around 0.13 resulting in a heterozygote frequency of about 23% and expected frequency of affected animals of about 2%. Although the sample size is relatively small and somewhat biased, these data are in general agreement with the protein-based surveys (Stokol, T. et al., *Res Vet Sci* 59:152–155 (1995); Brooks, M., *Probl In Vet Med* 4:636–646 (1992)), in that the allele frequency is substantial.

All data collected thus far indicate that this mutation accounts for essentially all of the von Willebrand's disease found in Scottish terriers. This result is consistent with the results found for other genetic diseases, defined at the molecular level, in various domestic animals (Shuster, D. E.

et al., *PNAS (USA)* 89:9225–9229 (1992); Rudolph, J. A. et al., *Nat Genet* 2:144–147 (1992); O'Brien, P. J. et al., *JAVMA* 203:842–851 (1993)). A likely explanation may be found in the pronounced founder effect that occurs in domestic animals, compared to most human and wild animal populations.

Published data using the protein-based factor assays have shown that, at least in several instances, obligate carriers have had factor levels that would lead to a diagnosis of "clear" of the disease allele. For example, in one study an obligate carrier had a factor level of 78% (Johnson, G. S. et al., *JAVMA* 176:1261–1263 (1980)). In another study, at least some of the obligate carriers had factor levels of 65% or greater (Brinkhous, K. M. et al., *Ann. New York Acad. Sci.* 370:191–203 (1981)). In addition, the number of animals that fall into an equivocal range can be substantial. In one study, 19% of Scottish terriers fell in this range (50–65% of the normal vWF antigen level) (Stokol, T. et al., *Res Vet Sci* 59:152–155 (1995)). Thus, although the protein-based tests have been useful, the certainty of the DNA-based test described herein should relieve the necessity of repeated testing and the variability associated with the protein-based assays.

The mutation is present in the pre-vWF part of the molecule. This part of the molecule is processed off prior to delivery of the mature protein into the plasma. This pre-portion of the molecule is important for the assembly of the mature vWF protein (Verwiej, L. et al., *EBMO J* 6:2885–2890 (1987); Wise, R. J. et al., *Cell* 52:229–236 (1988)). With the Scottish terrier frameshift vWD mutation, neither this pre-portion nor any of the mature factor is ever produced, in keeping with the fact that no factor has ever been detected in the blood of affected dogs.

The determination of the complete canine vWF cDNA sequence will have an impact upon the development of carrier tests for other breeds and other species as well. Currently, Shetland sheepdogs and Dutch Kooikers are known to have a significant amount of Type 3vWD (Brooks, M. et al., *JAVMA* 200:1123–1127 (1992); Slappendel, R. J., Vet-Q 17:S21–S22 (1995)). Type 3 vWD has occasionally be seen in other breeds as well (e.g., Johnson, G. S. et al., *JAVMA* 176:1261–1263 (1980)). All Type 3 vWD mutations described in humans to date have been found within the vWF gene itself. The availability of the canine sequence will make it easier to find the mutations in these breeds. In addition, at least some Type 1 mutations have been found within the human vWF gene, and thus Type 1 mutations may also be found within the vWF gene for breeds affected with that form of the disease. The availability of two divergent mammalian vWF cDNA sequences will also make it much easier to sequence the gene from other mammalian species using cross-species PCR methods (e.g., Venta et al., Biochem. Genet. (1996) in press).

The test described herein for the detection of the mutation in Scottish terriers may be performed on small amounts of DNA from any tissue. The tissues that are the least invasive to obtain are blood and buccal cells. For maximum convenience, a cheek swab as a source of DNA is preferred.

The foregoing discussion discloses and describes merely exemplary embodiments of the present invention. One skilled in the art will readily recognize from such discussion, and from the accompanying drawings, that various changes, modifications and variations can be made therein without departing from the spirit and scope of the invention.

All patents and other publications cited herein are expressly incorporated by reference.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 13

<210> SEQ ID NO 1
<211> LENGTH: 8802
<212> TYPE: DNA
<213> ORGANISM: Canine
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (203)...(8641)

<400> SEQUENCE: 1

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acaggggcca attaggatca atctttttc tttcttttt taaaaaaaaa a attcttccc     120

actttgcaca cggacagtag tacataccag tagctctctg cgaggacggt g atcactaat     180

catttctcct gcttcgtggc ag atg agt cct acc aga ct t gtg agg gtg ctg      232
                        Met Ser Pro Thr Arg Leu V al Arg Val Leu
                         1               5                  10

ctg gct ctg gcc ctc atc ttg cca ggg aaa c tt tgt aca aaa ggg act       280
Leu Ala Leu Ala Leu Ile Leu Pro Gly Lys L eu Cys Thr Lys Gly Thr
                15                  20                  25

gtt gga agg tca tcg atg gcc cga tgt agc c tt ctc gga ggt gac ttc       328
Val Gly Arg Ser Ser Met Ala Arg Cys Ser L eu Leu Gly Gly Asp Phe
            30                  35                  40

atc aac acc ttt gat gag agc atg tac agc t tt gcg gga gat tgc agt       376
Ile Asn Thr Phe Asp Glu Ser Met Tyr Ser P he Ala Gly Asp Cys Ser
        45                  50                  55
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tac ctc ctg gct ggg gac tgc cag gaa cac t cc atc tca ctt atc ggg      424
Tyr Leu Leu Ala Gly Asp Cys Gln Glu His S er Ile Ser Leu Ile Gly
    60                  65                  70

ggt ttc caa aat gac aaa aga gtg agc ctc t cc gtg tat ctc gga gaa      472
Gly Phe Gln Asn Asp Lys Arg Val Ser Leu S er Val Tyr Leu Gly Glu
 75                  80                  85                  90

ttt ttc gac att cat ttg ttt gtc aat ggt a cc atg ctg cag ggg acc      520
Phe Phe Asp Ile His Leu Phe Val Asn Gly T hr Met Leu Gln Gly Thr
                95                 100                 105

caa agc atc tcc atg ccc tac gcc tcc aat g gg ctg tat cta gag gcc      568
Gln Ser Ile Ser Met Pro Tyr Ala Ser Asn G ly Leu Tyr Leu Glu Ala
            110                 115                 120

gag gct ggc tac tac aag ctg tcc agt gag g cc tac ggc ttt gtg gcc      616
Glu Ala Gly Tyr Tyr Lys Leu Ser Ser Glu A la Tyr Gly Phe Val Ala
        125                 130                 135

aga att gat ggc aat ggc aac ttt caa gtc c tg ctg tca gac aga tac      664
Arg Ile Asp Gly Asn Gly Asn Phe Gln Val L eu Leu Ser Asp Arg Tyr
    140                 145                 150

ttc aac aag acc tgt ggg ctg tgt ggc aac t tt aat atc ttt gct gag      712
Phe Asn Lys Thr Cys Gly Leu Cys Gly Asn P he Asn Ile Phe Ala Glu
155                 160                 165                 170

gat gac ttc aag act caa gaa ggg acg ttg a ct tcg gac ccc tat gac      760
Asp Asp Phe Lys Thr Gln Glu Gly Thr Leu T hr Ser Asp Pro Tyr Asp
                175                 180                 185

ttt gcc aac tcc tgg gcc ctg agc agt ggg g aa caa cgg tgc aaa cgg      808
Phe Ala Asn Ser Trp Ala Leu Ser Ser Gly G lu Gln Arg Cys Lys Arg
            190                 195                 200

gtg tcc cct ccc agc agc cca tgc aat gtc t cc tct gat gaa gtg cag      856
Val Ser Pro Pro Ser Ser Pro Cys Asn Val S er Ser Asp Glu Val Gln
        205                 210                 215

cag gtc ctg tgg gag cag tgc cag ctc ctg a ag agt gcc tcg gtg ttt      904
Gln Val Leu Trp Glu Gln Cys Gln Leu Leu L ys Ser Ala Ser Val Phe
    220                 225                 230

gcc cgc tgc cac ccg ctg gtg gac cct gag c ct ttt gtc gcc ctg tgt      952
Ala Arg Cys His Pro Leu Val Asp Pro Glu P ro Phe Val Ala Leu Cys
235                 240                 245                 250

gaa agg act ctg tgc acc tgt gtc cag ggg a tg gag tgc cct tgt gcg     1000
Glu Arg Thr Leu Cys Thr Cys Val Gln Gly M et Glu Cys Pro Cys Ala
                255                 260                 265

gtc ctc ctg gag tac gcc cgg gcc tgt gcc c ag cag ggg att gtc ttg     1048
Val Leu Leu Glu Tyr Ala Arg Ala Cys Ala G ln Gln Gly Ile Val Leu
            270                 275                 280

tac ggc tgg acc gac cac agc gtc tgc cga c ca gca tgc cct gct ggc     1096
Tyr Gly Trp Thr Asp His Ser Val Cys Arg P ro Ala Cys Pro Ala Gly
        285                 290                 295

atg gag tac aag gag tgc gtg tcc cct tgc a cc aga act tgc cag agc     1144
Met Glu Tyr Lys Glu Cys Val Ser Pro Cys T hr Arg Thr Cys Gln Ser
    300                 305                 310

ctt cat gtc aaa gaa gtg tgt cag gag caa t gt gta gat ggc tgc agc     1192
Leu His Val Lys Glu Val Cys Gln Glu Gln C ys Val Asp Gly Cys Ser
315                 320                 325                 330

tgc ccc gag ggc cag ctc ctg gat gaa ggc c ac tgc gtg gga agt gct     1240
Cys Pro Glu Gly Gln Leu Leu Asp Glu Gly H is Cys Val Gly Ser Ala
                335                 340                 345

gag tgt tcc tgt gtg cat gct ggg caa cgg t ac cct ccg ggc gcc tcc     1288
Glu Cys Ser Cys Val His Ala Gly Gln Arg T yr Pro Pro Gly Ala Ser
            350                 355                 360

ctc tta cag gac tgc cac acc tgc att tgc c ga aat agc ctg tgg atc     1336
Leu Leu Gln Asp Cys His Thr Cys Ile Cys A rg Asn Ser Leu Trp Ile
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        365                 370                 375

tgc agc aat gaa gaa tgc cca ggc gag tgt c tg gtc aca gga cag tcc     1384
Cys Ser Asn Glu Glu Cys Pro Gly Glu Cys L eu Val Thr Gly Gln Ser
    380                 385                 390

cac ttc aag agc ttc gac aac agg tac ttc a cc ttc agt ggg gtc tgc     1432
His Phe Lys Ser Phe Asp Asn Arg Tyr Phe T hr Phe Ser Gly Val Cys
395                 400                 405                 410

cac tac ctg ctg gcc cag gac tgc cag gac c ac aca ttc tct gtt gtc     1480
His Tyr Leu Leu Ala Gln Asp Cys Gln Asp H is Thr Phe Ser Val Val
                415                 420                 425

ata gag act gtc cag tgt gcc gat gac ctg g at gct gtc tgc acc cgc     1528
Ile Glu Thr Val Gln Cys Ala Asp Asp Leu A sp Ala Val Cys Thr Arg
            430                 435                 440

tcg gtc acc gtc cgc ctg cct gga cat cac a ac agc ctt gtg aag ctg     1576
Ser Val Thr Val Arg Leu Pro Gly His His A sn Ser Leu Val Lys Leu
        445                 450                 455

aag aat ggg gga gga gtc tcc atg gat ggc c ag gat atc cag att cct     1624
Lys Asn Gly Gly Gly Val Ser Met Asp Gly G ln Asp Ile Gln Ile Pro
    460                 465                 470

ctc ctg caa ggt gac ctc cgc atc cag cac a cc gtg atg gcc tcc gtg     1672
Leu Leu Gln Gly Asp Leu Arg Ile Gln His T hr Val Met Ala Ser Val
475                 480                 485                 490

cgc ctc agc tac ggg gag gac ctg cag atg g at tcg gac gtc cgg ggc     1720
Arg Leu Ser Tyr Gly Glu Asp Leu Gln Met A sp Ser Asp Val Arg Gly
                495                 500                 505

agg cta ctg gtg acg ctg tac ccc gcc tac g cg ggg aag acg tgc ggc     1768
Arg Leu Leu Val Thr Leu Tyr Pro Ala Tyr A la Gly Lys Thr Cys Gly
            510                 515                 520

cgt ggc ggg aac tac aac ggc aac cgg ggg g ac gac ttc gtg acg ccc     1816
Arg Gly Gly Asn Tyr Asn Gly Asn Arg Gly A sp Asp Phe Val Thr Pro
        525                 530                 535

gca ggc ctg gcg gag ccc ctg gtg gag gac t tc ggg aac gcc tgg aag     1864
Ala Gly Leu Ala Glu Pro Leu Val Glu Asp P he Gly Asn Ala Trp Lys
    540                 545                 550

ctg ctc ggg gcc tgc gag aac ctg cag aag c ag cac cgc gat ccc tgc     1912
Leu Leu Gly Ala Cys Glu Asn Leu Gln Lys G ln His Arg Asp Pro Cys
555                 560                 565                 570

agc ctc aac ccg cgc cag gcc agg ttt gcg g ag gag gcg tgc gcg ctg     1960
Ser Leu Asn Pro Arg Gln Ala Arg Phe Ala G lu Glu Ala Cys Ala Leu
                575                 580                 585

ctg acg tcc tcg aag ttc gag ccc tgc cac c ga gcg gtg ggt cct cag     2008
Leu Thr Ser Ser Lys Phe Glu Pro Cys His A rg Ala Val Gly Pro Gln
            590                 595                 600

ccc tac gtg cag aac tgc ctc tac gac gtc t gc tcc tgc tcc gac ggc     2056
Pro Tyr Val Gln Asn Cys Leu Tyr Asp Val C ys Ser Cys Ser Asp Gly
        605                 610                 615

aga gac tgt ctt tgc agc gcc gtg gcc aac t ac gcc gca gcc gtg gcc     2104
Arg Asp Cys Leu Cys Ser Ala Val Ala Asn T yr Ala Ala Ala Val Ala
    620                 625                 630

cgg agg ggc gtg cac atc gcg tgg cgg gag c cg ggc ttc tgt gcg ctg     2152
Arg Arg Gly Val His Ile Ala Trp Arg Glu P ro Gly Phe Cys Ala Leu
635                 640                 645                 650

agc tgc ccc cag ggc cag gtg tac ctg cag t gt ggg acc ccc tgc aac     2200
Ser Cys Pro Gln Gly Gln Val Tyr Leu Gln C ys Gly Thr Pro Cys Asn
                655                 660                 665

atg acc tgt ctc tcc ctc tct tac ccg gag g ag gac tgc aat gag gtc     2248
Met Thr Cys Leu Ser Leu Ser Tyr Pro Glu G lu Asp Cys Asn Glu Val
            670                 675                 680

tgc ttg gaa agc tgc ttc tcc ccc cca ggg c tg tac ctg gat gag agg     2296
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Cys Leu Glu Ser Cys Phe Ser Pro Pro Gly L eu Tyr Leu Asp Glu Arg
        685                 690                 695

gga gat tgt gtg ccc aag gct cag tgt ccc t gt tac tat gat ggt gag     2344
Gly Asp Cys Val Pro Lys Ala Gln Cys Pro C ys Tyr Tyr Asp Gly Glu
    700                 705                 710

atc ttt cag ccc gaa gac atc ttc tca gac c at cac acc atg tgc tac     2392
Ile Phe Gln Pro Glu Asp Ile Phe Ser Asp H is His Thr Met Cys Tyr
715                 720                 725                 730

tgt gag gat ggc ttc atg cac tgt acc aca a gt gga ggc ctg gga agc     2440
Cys Glu Asp Gly Phe Met His Cys Thr Thr S er Gly Gly Leu Gly Ser
                735                 740                 745

ctg ctg ccc aac ccg gtg ctc agc agc ccc c gg tgt cac cgc agc aaa     2488
Leu Leu Pro Asn Pro Val Leu Ser Ser Pro A rg Cys His Arg Ser Lys
            750                 755                 760

agg agc ctg tcc tgt cgg ccc ccc atg gtc a ag ttg gtg tgt ccc gct     2536
Arg Ser Leu Ser Cys Arg Pro Pro Met Val L ys Leu Val Cys Pro Ala
        765                 770                 775

gat aac ccg agg gct gaa gga ctg gag tgt g cc aaa acc tgc cag aac     2584
Asp Asn Pro Arg Ala Glu Gly Leu Glu Cys A la Lys Thr Cys Gln Asn
    780                 785                 790

tat gac ctg cag tgc atg agc aca ggc tgt g tc tcc ggc tgc ctc tgc     2632
Tyr Asp Leu Gln Cys Met Ser Thr Gly Cys V al Ser Gly Cys Leu Cys
795                 800                 805                 810

ccg cag ggc atg gtc cgg cat gaa aac agg t gt gtg gcg ctg gaa aga     2680
Pro Gln Gly Met Val Arg His Glu Asn Arg C ys Val Ala Leu Glu Arg
                815                 820                 825

tgt ccc tgc ttc cac caa ggc caa gag tac g cc cca gga gaa acc gtg     2728
Cys Pro Cys Phe His Gln Gly Gln Glu Tyr A la Pro Gly Glu Thr Val
            830                 835                 840

aaa att gac tgc aac act tgt gtc tgt cgg g ac cgg aag tgg acc tgc     2776
Lys Ile Asp Cys Asn Thr Cys Val Cys Arg A sp Arg Lys Trp Thr Cys
        845                 850                 855

aca gac cat gtg tgt gat gcc act tgc tct g cc atc ggc atg gcg cac     2824
Thr Asp His Val Cys Asp Ala Thr Cys Ser A la Ile Gly Met Ala His
    860                 865                 870

tac ctc acc ttc gac gga ctc aag tac ctg t tc cct ggg gag tgc cag     2872
Tyr Leu Thr Phe Asp Gly Leu Lys Tyr Leu P he Pro Gly Glu Cys Gln
875                 880                 885                 890

tat gtt ctg gtg cag gat tac tgc ggc agt a ac cct ggg acc tta cgg     2920
Tyr Val Leu Val Gln Asp Tyr Cys Gly Ser A sn Pro Gly Thr Leu Arg
                895                 900                 905

atc ctg gtg ggg aac gag ggg tgc agc tac c cc tca gtg aaa tgc aag     2968
Ile Leu Val Gly Asn Glu Gly Cys Ser Tyr P ro Ser Val Lys Cys Lys
            910                 915                 920

aag cgg gtc acc atc ctg gtg gaa gga gga g ag att gaa ctg ttt gat     3016
Lys Arg Val Thr Ile Leu Val Glu Gly Gly G lu Ile Glu Leu Phe Asp
        925                 930                 935

ggg gag gtg aat gtg aag aaa ccc atg aag g at gag act cac ttt gag     3064
Gly Glu Val Asn Val Lys Lys Pro Met Lys A sp Glu Thr His Phe Glu
    940                 945                 950

gtg gta gag tct ggt cag tac gtc att ctg c tg ctg ggc aag gca ctc     3112
Val Val Glu Ser Gly Gln Tyr Val Ile Leu L eu Leu Gly Lys Ala Leu
955                 960                 965                 970

tct gtg gtc tgg gac cac cgc ctg agc atc t ct gtg acc ctg aag cgg     3160
Ser Val Val Trp Asp His Arg Leu Ser Ile S er Val Thr Leu Lys Arg
                975                 980                 985

aca tac cag gag cag gtg tgt ggc ctg tgt g gg aat ttt gat  ggc atc     3208
Thr Tyr Gln Glu Gln Val Cys Gly Leu Cys G ly Asn Phe Asp  Gly Ile
            990                 995                1000
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cag aac aat  gat ttc acc agc agc  agc ctc caa ata gaa  gaa gac cct  3256
Gln Asn Asn  Asp Phe Thr Ser Ser  Ser Leu Gln Ile Glu  Glu Asp Pro
        1005                 1010                 1015

gtg gac  ttt ggg aat tcc tgg  aaa gtg aac ccg cag  tgt gcc gac acc  3304
Val Asp  Phe Gly Asn Ser Trp  Lys Val Asn Pro Gln  Cys Ala Asp Thr
    1020                 1025                 1030

aag  aaa gta cca ctg gac  tca tcc cct gcc gtc  tgc cac aac aac atc  3352
Lys  Lys Val Pro Leu Asp  Ser Ser Pro Ala Val  Cys His Asn Asn Ile
1035                 1040                 1045                 1050

atg aag cag acg atg  gtg gat tcc tcc tgc  agg atc ctc acc agt  gat  3400
Met Lys Gln Thr Met  Val Asp Ser Ser Cys  Arg Ile Leu Thr Ser  Asp
                1055                 1060                 1065

att ttc cag gac  tgc aac agg ctg gtg  gac cct gag cca ttc  ctg gac  3448
Ile Phe Gln Asp  Cys Asn Arg Leu Val  Asp Pro Glu Pro Phe  Leu Asp
            1070                 1075                 1080

att tgc atc  tac gac act tgc tcc  tgt gag tcc att ggg  gac tgc acc  3496
Ile Cys Ile  Tyr Asp Thr Cys Ser  Cys Glu Ser Ile Gly  Asp Cys Thr
        1085                 1090                 1095

tgc ttc  tgt gac acc att gct  gct tac gcc cac gtc  tgt gcc cag cat  3544
Cys Phe  Cys Asp Thr Ile Ala  Ala Tyr Ala His Val  Cys Ala Gln His
    1100                 1105                 1110

ggc  aag gtg gta gcc tgg  agg aca gcc aca ttc  tgt ccc cag aat tgc  3592
Gly  Lys Val Val Ala Trp  Arg Thr Ala Thr Phe  Cys Pro Gln Asn Cys
1115                 1120                 1125                 1130

gag gag cgg aat ctc  cac gag aat ggg tat  gag tgt gag tgg cgc  tat  3640
Glu Glu Arg Asn Leu  His Glu Asn Gly Tyr  Glu Cys Glu Trp Arg  Tyr
                1135                 1140                 1145

aac agc tgt gcc  cct gcc tgt ccc atc  acg tgc cag cac ccc  gag cca  3688
Asn Ser Cys Ala  Pro Ala Cys Pro Ile  Thr Cys Gln His Pro  Glu Pro
            1150                 1155                 1160

ctg gca tgc  cct gta cag tgt gtt  gaa ggt tgc cat gcg  cac tgc cct  3736
Leu Ala Cys  Pro Val Gln Cys Val  Glu Gly Cys His Ala  His Cys Pro
        1165                 1170                 1175

cca ggg  aaa atc ctg gat gag  ctt ttg cag acc tgc  atc gac cct gaa  3784
Pro Gly  Lys Ile Leu Asp Glu  Leu Leu Gln Thr Cys  Ile Asp Pro Glu
    1180                 1185                 1190

gac  tgt cct gtg tgt gag  gtg gct ggt cgt cgc  ttg gcc cca gga aag  3832
Asp  Cys Pro Val Cys Glu  Val Ala Gly Arg Arg  Leu Ala Pro Gly Lys
1195                 1200                 1205                 1210

aaa atc atc ttg aac  ccc agt gac cct gag  cac tgc caa att tgt  aat  3880
Lys Ile Ile Leu Asn  Pro Ser Asp Pro Glu  His Cys Gln Ile Cys  Asn
                1215                 1220                 1225

tgt gat ggt gtc  aac ttc acc tgt aag  gcc tgc aga gaa ccc  gga agt  3928
Cys Asp Gly Val  Asn Phe Thr Cys Lys  Ala Cys Arg Glu Pro  Gly Ser
            1230                 1235                 1240

gtt gtg gtg  ccc ccc aca gat ggc  ccc att ggc tct acc  acc tcg tat  3976
Val Val Val  Pro Pro Thr Asp Gly  Pro Ile Gly Ser Thr  Thr Ser Tyr
        1245                 1250                 1255

gtg gag  gac acg tcg gag ccg  ccc ctc cat gac ttc  cac tgc agc agg  4024
Val Glu  Asp Thr Ser Glu Pro  Pro Leu His Asp Phe  His Cys Ser Arg
    1260                 1265                 1270

ctt  ctg gac ctg gtt ttc  ctg ctg gat ggc tcc  tcc aag ctg tct gag  4072
Leu  Leu Asp Leu Val Phe  Leu Leu Asp Gly Ser  Ser Lys Leu Ser Glu
1275                 1280                 1285                 1290

gac gag ttt gaa gtg  ctg aag gtc ttt gtg  gtg ggt atg atg gag  cat  4120
Asp Glu Phe Glu Val  Leu Lys Val Phe Val  Val Gly Met Met Glu  His
                1295                 1300                 1305

ctg cac atc tcc  cag aag cgg atc cgc  gtg gct gtg gtg gag  tac cac  4168
Leu His Ile Ser  Gln Lys Arg Ile Arg  Val Ala Val Val Glu  Tyr His
            1310                 1315                 1320
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gac ggc tcc  cac gcc tac atc gag  ctc aag gac cgg aag  cga ccc tca  4216
Asp Gly Ser  His Ala Tyr Ile Glu  Leu Lys Asp Arg Lys  Arg Pro Ser
        1325                 1330                 1335

gag ctg  cgg cgc atc acc agc  cag gtg aag tac gcg  ggc agc gag gtg  4264
Glu Leu  Arg Arg Ile Thr Ser  Gln Val Lys Tyr Ala  Gly Ser Glu Val
    1340                 1345                 1350

gcc  tcc acc agt gag gtc  tta aag tac acg ctg  ttc cag atc ttt ggc  4312
Ala  Ser Thr Ser Glu Val  Leu Lys Tyr Thr Leu  Phe Gln Ile Phe Gly
1355                 1360                 1365                 1370

aag atc gac cgc ccg  gaa gcg tct cgc att  gcc ctg ctc ctg atg  gcc  4360
Lys Ile Asp Arg Pro  Glu Ala Ser Arg Ile  Ala Leu Leu Leu Met  Ala
                1375                 1380                 1385

agc cag gag ccc  tca agg ctg gcc cgg  aat ttg gtc cgc tat  gtg cag  4408
Ser Gln Glu Pro  Ser Arg Leu Ala Arg  Asn Leu Val Arg Tyr  Val Gln
            1390                 1395                 1400

ggc ctg aag  aag aag aaa gtc att  gtc atc cct gtg ggc  atc ggg ccc  4456
Gly Leu Lys  Lys Lys Lys Val Ile  Val Ile Pro Val Gly  Ile Gly Pro
        1405                 1410                 1415

cac gcc  agc ctt aag cag atc  cac ctc ata gag aag  cag gcc cct gag  4504
His Ala  Ser Leu Lys Gln Ile  His Leu Ile Glu Lys  Gln Ala Pro Glu
    1420                 1425                 1430

aac  aag gcc ttt gtg ttc  agt ggt gtg gat gag  ttg gag cag cga agg  4552
Asn  Lys Ala Phe Val Phe  Ser Gly Val Asp Glu  Leu Glu Gln Arg Arg
1435                 1440                 1445                 1450

gat gag att atc aac  tac ctc tgt gac ctt  gcc ccc gaa gca cct  gcc  4600
Asp Glu Ile Ile Asn  Tyr Leu Cys Asp Leu  Ala Pro Glu Ala Pro  Ala
                1455                 1460                 1465

cct act cag cac  ccc cca atg gcc cag  gtc acg gtg ggt tcg  gag ctg  4648
Pro Thr Gln His  Pro Pro Met Ala Gln  Val Thr Val Gly Ser  Glu Leu
            1470                 1475                 1480

ttg ggg gtt  tca tct cca gga ccc  aaa agg aac tcc atg  gtc ctg gat  4696
Leu Gly Val  Ser Ser Pro Gly Pro  Lys Arg Asn Ser Met  Val Leu Asp
        1485                 1490                 1495

gtg gtg  ttt gtc ctg gaa ggg  tca gac aaa att ggt  gag gcc aac ttt  4744
Val Val  Phe Val Leu Glu Gly  Ser Asp Lys Ile Gly  Glu Ala Asn Phe
    1500                 1505                 1510

aac  aaa agc agg gag ttc  atg gag gag gtg att  cag cgg atg gac gtg  4792
Asn  Lys Ser Arg Glu Phe  Met Glu Glu Val Ile  Gln Arg Met Asp Val
1515                 1520                 1525                 1530

ggc cag gac agg atc  cac gtc aca gtg ctg  cag tac tcg tac atg  gtg  4840
Gly Gln Asp Arg Ile  His Val Thr Val Leu  Gln Tyr Ser Tyr Met  Val
                1535                 1540                 1545

acc gtg gag tac  acc ttc agc gag gcg  cag tcc aag ggc gag  gtc cta  4888
Thr Val Glu Tyr  Thr Phe Ser Glu Ala  Gln Ser Lys Gly Glu  Val Leu
            1550                 1555                 1560

cag cag gtg  cgg gat atc cga tac  cgg ggt ggc aac agg  acc aac act  4936
Gln Gln Val  Arg Asp Ile Arg Tyr  Arg Gly Gly Asn Arg  Thr Asn Thr
        1565                 1570                 1575

gga ctg  gcc ctg caa tac ctg  tcc gaa cac agc ttc  tcg gtc agc cag  4984
Gly Leu  Ala Leu Gln Tyr Leu  Ser Glu His Ser Phe  Ser Val Ser Gln
    1580                 1585                 1590

ggg  gac cgg gag cag gta  cct aac ctg gtc tac  atg gtc aca gga aac  5032
Gly  Asp Arg Glu Gln Val  Pro Asn Leu Val Tyr  Met Val Thr Gly Asn
1595                 1600                 1605                 1610

ccc gct tct gat gag  atc aag cgg atg cct  gga gac atc cag gtg  gtg  5080
Pro Ala Ser Asp Glu  Ile Lys Arg Met Pro  Gly Asp Ile Gln Val  Val
                1615                 1620                 1625

ccc atc ggg gtg  ggt cca cat gcc aat  gtg cag gag ctg gag  aag att  5128
Pro Ile Gly Val  Gly Pro His Ala Asn  Val Gln Glu Leu Glu  Lys Ile
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            1630                1635                1640

ggc tgg ccc  aat gcc ccc atc ctc  atc cat gac ttt gag  atg ctc cct  5176
Gly Trp Pro  Asn Ala Pro Ile Leu  Ile His Asp Phe Glu  Met Leu Pro
        1645                1650                1655

cga gag  gct cct gat ctg gtg  cta cag agg tgc tgc  tct gga gag ggg  5224
Arg Glu  Ala Pro Asp Leu Val  Leu Gln Arg Cys Cys  Ser Gly Glu Gly
    1660                1665                1670

ctg  cag atc ccc acc ctc  tcc ccc acc cca gat  tgc agc cag ccc ctg  5272
Leu  Gln Ile Pro Thr Leu  Ser Pro Thr Pro Asp  Cys Ser Gln Pro Leu
1675                1680                1685                1690

gat gtg gtc ctc ctc  ctg gat ggc tct tcc  agc att cca gct tct  tac  5320
Asp Val Val Leu Leu  Leu Asp Gly Ser Ser  Ser Ile Pro Ala Ser  Tyr
                1695                1700                1705

ttt gat gaa atg  aag agc ttc acc aag  gct ttt att tca aga  gct aat  5368
Phe Asp Glu Met  Lys Ser Phe Thr Lys  Ala Phe Ile Ser Arg  Ala Asn
            1710                1715                1720

ata ggg ccc  cgg ctc act caa gtg  tcg gtg ctg caa tat  gga agc atc  5416
Ile Gly Pro  Arg Leu Thr Gln Val  Ser Val Leu Gln Tyr  Gly Ser Ile
        1725                1730                1735

acc act  atc gat gtg cct tgg  aat gta gcc tat gag  aaa gtc cat tta  5464
Thr Thr  Ile Asp Val Pro Trp  Asn Val Ala Tyr Glu  Lys Val His Leu
    1740                1745                1750

ctg  agc ctt gtg gac ctc  atg cag cag gag gga  ggc ccc agc gaa att  5512
Leu  Ser Leu Val Asp Leu  Met Gln Gln Glu Gly  Gly Pro Ser Glu Ile
1755                1760                1765                1770

ggg gat gct ttg agc  ttt gcc gtg cga tat  gtc acc tca gaa gtc  cat  5560
Gly Asp Ala Leu Ser  Phe Ala Val Arg Tyr  Val Thr Ser Glu Val  His
                1775                1780                1785

ggt gcc agg ccc  gga gcc tcg aaa gcg  gtg gtt atc cta gtc  aca gat  5608
Gly Ala Arg Pro  Gly Ala Ser Lys Ala  Val Val Ile Leu Val  Thr Asp
            1790                1795                1800

gtc tcc gtg  gat tca gtg gat gct  gca gcc gag gcc gcc  aga tcc aac  5656
Val Ser Val  Asp Ser Val Asp Ala  Ala Ala Glu Ala Ala  Arg Ser Asn
        1805                1810                1815

cga gtg  aca gtg ttc ccc att  gga atc ggg gat cgg  tac agt gag gcc  5704
Arg Val  Thr Val Phe Pro Ile  Gly Ile Gly Asp Arg  Tyr Ser Glu Ala
    1820                1825                1830

cag  ctg agc agc ttg gca  ggc cca aag gct ggc  tcc aat atg gta agg  5752
Gln  Leu Ser Ser Leu Ala  Gly Pro Lys Ala Gly  Ser Asn Met Val Arg
1835                1840                1845                1850

ctc cag cga att gaa  gac ctc ccc acc gtg  gcc acc ctg gga aat  tcc  5800
Leu Gln Arg Ile Glu  Asp Leu Pro Thr Val  Ala Thr Leu Gly Asn  Ser
                1855                1860                1865

ttc ttc cac aag  ctg tgc tct ggg ttt  gat aga gtt tgc gtg  gat gag  5848
Phe Phe His Lys  Leu Cys Ser Gly Phe  Asp Arg Val Cys Val  Asp Glu
            1870                1875                1880

gat ggg aat  gag aag agg ccc ggg  gat gtc tgg acc ttg  cca gac cag  5896
Asp Gly Asn  Glu Lys Arg Pro Gly  Asp Val Trp Thr Leu  Pro Asp Gln
        1885                1890                1895

tgc cac  aca gtg act tgc ctg  cca gat ggc cag acc  ttg ctg aag agt  5944
Cys His  Thr Val Thr Cys Leu  Pro Asp Gly Gln Thr  Leu Leu Lys Ser
    1900                1905                1910

cat  cgg gtc aac tgt gac  cgg ggg cca agg cct  tcg tgc ccc aat ggc  5992
His  Arg Val Asn Cys Asp  Arg Gly Pro Arg Pro  Ser Cys Pro Asn Gly
1915                1920                1925                1930

cag ccc cct ctc agg  gta gag gag acc tgt  ggc tgc cgc tgg acc  tgt  6040
Gln Pro Pro Leu Arg  Val Glu Glu Thr Cys  Gly Cys Arg Trp Thr  Cys
                1935                1940                1945

ccc tgt gtg tgc  atg ggc agc tct acc  cgg cac atc gtg acc  ttt gat  6088
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Pro Cys Val Cys  Met Gly Ser Ser Thr  Arg His Ile Val Thr  Phe Asp
            1950                1955                1960

ggg cag aat  ttc aag ctg act ggc  agc tgt tcg tat gtc  cta ttt caa  6136
Gly Gln Asn  Phe Lys Leu Thr Gly  Ser Cys Ser Tyr Val  Leu Phe Gln
        1965                1970                1975

aac aag  gag cag gac ctg gag  gtg att ctc cag aat  ggt gcc tgc agc  6184
Asn Lys  Glu Gln Asp Leu Glu  Val Ile Leu Gln Asn  Gly Ala Cys Ser
    1980                1985                1990

cct  ggg gcg aag gag acc  tgc atg aaa tcc att  gag gtg aag cat gac  6232
Pro  Gly Ala Lys Glu Thr  Cys Met Lys Ser Ile  Glu Val Lys His Asp
1995                2000                2005                2010

ggc ctc tca gtt gag  ctc cac agt gac atg  cag atg aca gtg aat  ggg  6280
Gly Leu Ser Val Glu  Leu His Ser Asp Met  Gln Met Thr Val Asn  Gly
                2015                2020                2025

aga cta gtc tcc  atc cca tat gtg ggt  gga gac atg gaa gtc  aat gtt  6328
Arg Leu Val Ser  Ile Pro Tyr Val Gly  Gly Asp Met Glu Val  Asn Val
            2030                2035                2040

tat ggg acc  atc atg tat gag gtc  aga ttc aac cat ctt  ggc cac atc  6376
Tyr Gly Thr  Ile Met Tyr Glu Val  Arg Phe Asn His Leu  Gly His Ile
        2045                2050                2055

ttc aca  ttc acc ccc caa aac  aat gag ttc cag ctg  cag ctc agc ccc  6424
Phe Thr  Phe Thr Pro Gln Asn  Asn Glu Phe Gln Leu  Gln Leu Ser Pro
    2060                2065                2070

agg  acc ttt gct tcg aag  aca tat ggt ctc tgt  ggg atc tgt gat gag  6472
Arg  Thr Phe Ala Ser Lys  Thr Tyr Gly Leu Cys  Gly Ile Cys Asp Glu
2075                2080                2085                2090

aac gga gcc aat gac  ttc att ctg agg gat  ggg aca gtc acc aca  gac  6520
Asn Gly Ala Asn Asp  Phe Ile Leu Arg Asp  Gly Thr Val Thr Thr  Asp
                2095                2100                2105

tgg aag gca ctc  atc cag gaa tgg acc  gta cag cag ctt ggg  aag aca  6568
Trp Lys Ala Leu  Ile Gln Glu Trp Thr  Val Gln Gln Leu Gly  Lys Thr
            2110                2115                2120

tcc cag cct  gtc cat gag gag cag  tgt cct gtc tcc gaa  ttc ttc cac  6616
Ser Gln Pro  Val His Glu Glu Gln  Cys Pro Val Ser Glu  Phe Phe His
        2125                2130                2135

tgc cag  gtc ctc ctc tca gaa  ttg ttt gcc gag tgc  cac aag gtc ctc  6664
Cys Gln  Val Leu Leu Ser Glu  Leu Phe Ala Glu Cys  His Lys Val Leu
    2140                2145                2150

gct  cca gcc acc ttt tat  gcc atg tgc cag ccc  gac agt tgc cac ccg  6712
Ala  Pro Ala Thr Phe Tyr  Ala Met Cys Gln Pro  Asp Ser Cys His Pro
2155                2160                2165                2170

aag aaa gtg tgt gag  gcg att gcc ttg tat  gcc cac ctc tgt cgg  acc  6760
Lys Lys Val Cys Glu  Ala Ile Ala Leu Tyr  Ala His Leu Cys Arg  Thr
                2175                2180                2185

aaa ggg gtc tgt  gtg gac tgg agg agg  gcc aat ttc tgt gct  atg tca  6808
Lys Gly Val Cys  Val Asp Trp Arg Arg  Ala Asn Phe Cys Ala  Met Ser
            2190                2195                2200

tgt cca cca  tcc ctg gtg tac aac  cac tgt gag cat ggc  tgc cct cgg  6856
Cys Pro Pro  Ser Leu Val Tyr Asn  His Cys Glu His Gly  Cys Pro Arg
        2205                2210                2215

ctc tgt  gaa ggc aat aca agc  tcc tgt ggg gac caa  ccc tcg gaa ggc  6904
Leu Cys  Glu Gly Asn Thr Ser  Ser Cys Gly Asp Gln  Pro Ser Glu Gly
    2220                2225                2230

tgc  ttc tgc ccc cca aac  caa gtc atg ctg gaa  ggt agc tgt gtc ccc  6952
Cys  Phe Cys Pro Pro Asn  Gln Val Met Leu Glu  Gly Ser Cys Val Pro
2235                2240                2245                2250

gag gag gcc tgt acc  cag tgc atc agc gag  gat gga gtc cgg cac  cag  7000
Glu Glu Ala Cys Thr  Gln Cys Ile Ser Glu  Asp Gly Val Arg His  Gln
                2255                2260                2265
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ttc ctg gaa acc  tgg gtc cca gcc cac  cag cct tgc cag atc  tgc acg  7048
Phe Leu Glu Thr  Trp Val Pro Ala His  Gln Pro Cys Gln Ile  Cys Thr
            2270                2275                2280

tgc ctc agt  ggg cgg aag gtc aac  tgt acg ttg cag ccc  tgc ccc aca  7096
Cys Leu Ser  Gly Arg Lys Val Asn  Cys Thr Leu Gln Pro  Cys Pro Thr
        2285                2290                2295

gcc aaa  gct ccc acc tgt ggc  ccg tgt gaa gtg gcc  cgc ctc cgc cag  7144
Ala Lys  Ala Pro Thr Cys Gly  Pro Cys Glu Val Ala  Arg Leu Arg Gln
    2300                2305                2310

aac  gca gtg cag tgc tgc  ccg gag tac gag tgt  gtg tgt gac ctg gtg  7192
Asn  Ala Val Gln Cys Cys  Pro Glu Tyr Glu Cys  Val Cys Asp Leu Val
2315                2320                2325                2330

agc tgt gac ctg ccc  ccg gtg cct ccc tgc  gaa gat ggc ctc cag  atg  7240
Ser Cys Asp Leu Pro  Pro Val Pro Pro Cys  Glu Asp Gly Leu Gln  Met
                2335                2340                2345

acc ctg acc aat  cct ggc gag tgc aga  ccc aac ttc acc tgt  gcc tgc  7288
Thr Leu Thr Asn  Pro Gly Glu Cys Arg  Pro Asn Phe Thr Cys  Ala Cys
            2350                2355                2360

agg aag gat  gaa tgc aga cgg gag  tcc ccg ccc tct tgt  ccc ccg cac  7336
Arg Lys Asp  Glu Cys Arg Arg Glu  Ser Pro Pro Ser Cys  Pro Pro His
        2365                2370                2375

cgg acg  ccg gcc ctt cgg aag  act cag tgc tgt gat  gag tat gag tgt  7384
Arg Thr  Pro Ala Leu Arg Lys  Thr Gln Cys Cys Asp  Glu Tyr Glu Cys
    2380                2385                2390

gca  tgc aac tgt gtc aac  tcc acg gtg agc tgc  ccg ctt ggg tac ctg  7432
Ala  Cys Asn Cys Val Asn  Ser Thr Val Ser Cys  Pro Leu Gly Tyr Leu
2395                2400                2405                2410

gcc tcg gct gtc acc  aac gac tgt ggc tgc  acc aca aca acc tgc  ttc  7480
Ala Ser Ala Val Thr  Asn Asp Cys Gly Cys  Thr Thr Thr Thr Cys  Phe
                2415                2420                2425

cct gac aag gtg  tgt gtc cac cga ggc  acc atc tac cct gtg  ggc cag  7528
Pro Asp Lys Val  Cys Val His Arg Gly  Thr Ile Tyr Pro Val  Gly Gln
            2430                2435                2440

ttc tgg gag  gag gcc tgt gac gtg  tgc acc tgc acg gac  ttg gag gac  7576
Phe Trp Glu  Glu Ala Cys Asp Val  Cys Thr Cys Thr Asp  Leu Glu Asp
        2445                2450                2455

tct gtg  atg ggc ctg cgt gtg  gcc cag tgc tcc cag  aag ccc tgt gag  7624
Ser Val  Met Gly Leu Arg Val  Ala Gln Cys Ser Gln  Lys Pro Cys Glu
    2460                2465                2470

gac  aac tgc ctg tca ggc  ttc act tat gtc ctt  cat gaa ggc gag tgc  7672
Asp  Asn Cys Leu Ser Gly  Phe Thr Tyr Val Leu  His Glu Gly Glu Cys
2475                2480                2485                2490

tgt gga agg tgt ctg  cca tct gcc tgt gag  gtg gtc act ggt tca  cca  7720
Cys Gly Arg Cys Leu  Pro Ser Ala Cys Glu  Val Val Thr Gly Ser  Pro
                2495                2500                2505

cgg ggc gac gcc  cag tct cac tgg aag  aat gtt ggc tct cac  tgg gcc  7768
Arg Gly Asp Ala  Gln Ser His Trp Lys  Asn Val Gly Ser His  Trp Ala
            2510                2515                2520

tcc cct gac  aac ccc tgc ctc atc  aat gag tgt gtc cga  gtg aag gaa  7816
Ser Pro Asp  Asn Pro Cys Leu Ile  Asn Glu Cys Val Arg  Val Lys Glu
        2525                2530                2535

gag gtc  ttt gtg caa cag agg  aat gtc tcc tgc ccc  cag ctg aat gtc  7864
Glu Val  Phe Val Gln Gln Arg  Asn Val Ser Cys Pro  Gln Leu Asn Val
    2540                2545                2550

ccc  acc tgc ccc acg ggc  ttc cag ctg agc tgt  aag acc tca gag tgt  7912
Pro  Thr Cys Pro Thr Gly  Phe Gln Leu Ser Cys  Lys Thr Ser Glu Cys
2555                2560                2565                2570

tgt ccc acc tgt cac  tgc gag ccc ctg gag  gcc tgc ttg ctc aat  ggt  7960
Cys Pro Thr Cys His  Cys Glu Pro Leu Glu  Ala Cys Leu Leu Asn  Gly
                2575                2580                2585
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acc atc att ggg  ccg ggg aaa agt ctg  atg att gat gtg tgt  aca acc  8008
Thr Ile Ile Gly  Pro Gly Lys Ser Leu  Met Ile Asp Val Cys  Thr Thr
            2590                2595                2600

tgc cgc tgc  acc gtg ccg gtg gga  gtc atc tct gga ttc  aag ctg gag  8056
Cys Arg Cys  Thr Val Pro Val Gly  Val Ile Ser Gly Phe  Lys Leu Glu
        2605                2610                2615

ggc agg  aag acc acc tgt gag  gca tgc ccc ctg ggt  tat aag gaa gag  8104
Gly Arg  Lys Thr Thr Cys Glu  Ala Cys Pro Leu Gly  Tyr Lys Glu Glu
    2620                2625                2630

aag  aac caa ggt gaa tgc  tgt ggg aga tgt ctg  cct ata gct tgc acc  8152
Lys  Asn Gln Gly Glu Cys  Cys Gly Arg Cys Leu  Pro Ile Ala Cys Thr
2635                2640                2645                2650

att cag cta aga gga  gga cag atc atg aca  ctg aag cgt gat gag  act  8200
Ile Gln Leu Arg Gly  Gly Gln Ile Met Thr  Leu Lys Arg Asp Glu  Thr
                2655                2660                2665

atc cag gat ggc  tgt gac agt cac ttc  tgc aag gtc aat gaa  aga gga  8248
Ile Gln Asp Gly  Cys Asp Ser His Phe  Cys Lys Val Asn Glu  Arg Gly
            2670                2675                2680

gag tac atc  tgg gag aag aga gtc  acg ggt tgc cca cct  ttc gat gaa  8296
Glu Tyr Ile  Trp Glu Lys Arg Val  Thr Gly Cys Pro Pro  Phe Asp Glu
        2685                2690                2695

cac aag  tgt ctg gct gag gga  gga aaa atc atg aaa  att cca ggc acc  8344
His Lys  Cys Leu Ala Glu Gly  Gly Lys Ile Met Lys  Ile Pro Gly Thr
    2700                2705                2710

tgc  tgt gac aca tgt gag  gag cca gaa tgc aag  gat atc att gcc aag  8392
Cys  Cys Asp Thr Cys Glu  Glu Pro Glu Cys Lys  Asp Ile Ile Ala Lys
2715                2720                2725                2730

ctg cag cgt gtc aaa  gtg gga gac tgt aag  tct gaa gag gaa gtg  gac  8440
Leu Gln Arg Val Lys  Val Gly Asp Cys Lys  Ser Glu Glu Glu Val  Asp
                2735                2740                2745

att cat tac tgt  gag ggt aaa tgt gcc  agc aaa gcc gtg tac  tcc atc  8488
Ile His Tyr Cys  Glu Gly Lys Cys Ala  Ser Lys Ala Val Tyr  Ser Ile
            2750                2755                2760

cac atg gag  gat gtg cag gac cag  tgc tcc tgc tgc tcg  ccc acc cag  8536
His Met Glu  Asp Val Gln Asp Gln  Cys Ser Cys Cys Ser  Pro Thr Gln
        2765                2770                2775

acg gag  ccc atg cag gtg gcc  ctg cgc tgc acc aat  ggc tcc ctc atc  8584
Thr Glu  Pro Met Gln Val Ala  Leu Arg Cys Thr Asn  Gly Ser Leu Ile
    2780                2785                2790

tac  cat gag atc ctc aat  gcc atc gaa tgc agg  tgt tcc ccc agg aag  8632
Tyr  His Glu Ile Leu Asn  Ala Ile Glu Cys Arg  Cys Ser Pro Arg Lys
2795                2800                2805                2810

tgc agc aag tgaggccact gcctggatgc tactgtcgcc tgccttac cc             8681
Cys Ser Lys

gacctcactg gactggccag agtgctgctc agtcctcctc agtcctcctc c tgctctgct   8741

cttgtgcttc ctgatcccac aataaaggtc aatctttcac cttgaaaaaa a aaaaaaaaa   8801

a                                                                    8802


<210> SEQ ID NO 2
<211> LENGTH: 2813
<212> TYPE: PRT
<213> ORGANISM: Canine

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Met Ser Pro Thr Arg Leu Val Arg Val Leu L eu Ala Leu Ala Leu Ile
 1               5                  10                  15

Leu Pro Gly Lys Leu Cys Thr Lys Gly Thr V al Gly Arg Ser Ser Met
            20                  25                  30
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Ala Arg Cys Ser Leu Leu Gly Gly Asp Phe I le Asn Thr Phe Asp Glu
        35                  40                  45

Ser Met Tyr Ser Phe Ala Gly Asp Cys Ser T yr Leu Leu Ala Gly Asp
    50                  55                  60

Cys Gln Glu His Ser Ile Ser Leu Ile Gly G ly Phe Gln Asn Asp Lys
65                  70                  75                  80

Arg Val Ser Leu Ser Val Tyr Leu Gly Glu P he Phe Asp Ile His Leu
                85                  90                  95

Phe Val Asn Gly Thr Met Leu Gln Gly Thr G ln Ser Ile Ser Met Pro
            100                 105                 110

Tyr Ala Ser Asn Gly Leu Tyr Leu Glu Ala G lu Ala Gly Tyr Tyr Lys
        115                 120                 125

Leu Ser Ser Glu Ala Tyr Gly Phe Val Ala A rg Ile Asp Gly Asn Gly
    130                 135                 140

Asn Phe Gln Val Leu Leu Ser Asp Arg Tyr P he Asn Lys Thr Cys Gly
145                 150                 155                 160

Leu Cys Gly Asn Phe Asn Ile Phe Ala Glu A sp Asp Phe Lys Thr Gln
                165                 170                 175

Glu Gly Thr Leu Thr Ser Asp Pro Tyr Asp P he Ala Asn Ser Trp Ala
            180                 185                 190

Leu Ser Ser Gly Glu Gln Arg Cys Lys Arg V al Ser Pro Pro Ser Ser
        195                 200                 205

Pro Cys Asn Val Ser Ser Asp Glu Val Gln G ln Val Leu Trp Glu Gln
    210                 215                 220

Cys Gln Leu Leu Lys Ser Ala Ser Val Phe A la Arg Cys His Pro Leu
225                 230                 235                 240

Val Asp Pro Glu Pro Phe Val Ala Leu Cys G lu Arg Thr Leu Cys Thr
                245                 250                 255

Cys Val Gln Gly Met Glu Cys Pro Cys Ala V al Leu Leu Glu Tyr Ala
            260                 265                 270

Arg Ala Cys Ala Gln Gln Gly Ile Val Leu T yr Gly Trp Thr Asp His
        275                 280                 285

Ser Val Cys Arg Pro Ala Cys Pro Ala Gly M et Glu Tyr Lys Glu Cys
    290                 295                 300

Val Ser Pro Cys Thr Arg Thr Cys Gln Ser L eu His Val Lys Glu Val
305                 310                 315                 320

Cys Gln Glu Gln Cys Val Asp Gly Cys Ser C ys Pro Glu Gly Gln Leu
                325                 330                 335

Leu Asp Glu Gly His Cys Val Gly Ser Ala G lu Cys Ser Cys Val His
            340                 345                 350

Ala Gly Gln Arg Tyr Pro Pro Gly Ala Ser L eu Leu Gln Asp Cys His
        355                 360                 365

Thr Cys Ile Cys Arg Asn Ser Leu Trp Ile C ys Ser Asn Glu Glu Cys
    370                 375                 380

Pro Gly Glu Cys Leu Val Thr Gly Gln Ser H is Phe Lys Ser Phe Asp
385                 390                 395                 400

Asn Arg Tyr Phe Thr Phe Ser Gly Val Cys H is Tyr Leu Leu Ala Gln
                405                 410                 415

Asp Cys Gln Asp His Thr Phe Ser Val Val I le Glu Thr Val Gln Cys
            420                 425                 430

Ala Asp Asp Leu Asp Ala Val Cys Thr Arg S er Val Thr Val Arg Leu
        435                 440                 445
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Pro Gly His His Asn Ser Leu Val Lys Leu L ys Asn Gly Gly Gly Val
    450                 455                 460

Ser Met Asp Gly Gln Asp Ile Gln Ile Pro L eu Leu Gln Gly Asp Leu
465                 470                 475                 480

Arg Ile Gln His Thr Val Met Ala Ser Val A rg Leu Ser Tyr Gly Glu
                485                 490                 495

Asp Leu Gln Met Asp Ser Asp Val Arg Gly A rg Leu Leu Val Thr Leu
            500                 505                 510

Tyr Pro Ala Tyr Ala Gly Lys Thr Cys Gly A rg Gly Gly Asn Tyr Asn
        515                 520                 525

Gly Asn Arg Gly Asp Asp Phe Val Thr Pro A la Gly Leu Ala Glu Pro
    530                 535                 540

Leu Val Glu Asp Phe Gly Asn Ala Trp Lys L eu Leu Gly Ala Cys Glu
545                 550                 555                 560

Asn Leu Gln Lys Gln His Arg Asp Pro Cys S er Leu Asn Pro Arg Gln
                565                 570                 575

Ala Arg Phe Ala Glu Glu Ala Cys Ala Leu L eu Thr Ser Ser Lys Phe
            580                 585                 590

Glu Pro Cys His Arg Ala Val Gly Pro Gln P ro Tyr Val Gln Asn Cys
        595                 600                 605

Leu Tyr Asp Val Cys Ser Cys Ser Asp Gly A rg Asp Cys Leu Cys Ser
    610                 615                 620

Ala Val Ala Asn Tyr Ala Ala Ala Val Ala A rg Arg Gly Val His Ile
625                 630                 635                 640

Ala Trp Arg Glu Pro Gly Phe Cys Ala Leu S er Cys Pro Gln Gly Gln
                645                 650                 655

Val Tyr Leu Gln Cys Gly Thr Pro Cys Asn M et Thr Cys Leu Ser Leu
            660                 665                 670

Ser Tyr Pro Glu Glu Asp Cys Asn Glu Val C ys Leu Glu Ser Cys Phe
        675                 680                 685

Ser Pro Pro Gly Leu Tyr Leu Asp Glu Arg G ly Asp Cys Val Pro Lys
    690                 695                 700

Ala Gln Cys Pro Cys Tyr Tyr Asp Gly Glu I le Phe Gln Pro Glu Asp
705                 710                 715                 720

Ile Phe Ser Asp His His Thr Met Cys Tyr C ys Glu Asp Gly Phe Met
                725                 730                 735

His Cys Thr Thr Ser Gly Gly Leu Gly Ser L eu Leu Pro Asn Pro Val
            740                 745                 750

Leu Ser Ser Pro Arg Cys His Arg Ser Lys A rg Ser Leu Ser Cys Arg
        755                 760                 765

Pro Pro Met Val Lys Leu Val Cys Pro Ala A sp Asn Pro Arg Ala Glu
    770                 775                 780

Gly Leu Glu Cys Ala Lys Thr Cys Gln Asn T yr Asp Leu Gln Cys Met
785                 790                 795                 800

Ser Thr Gly Cys Val Ser Gly Cys Leu Cys P ro Gln Gly Met Val Arg
                805                 810                 815

His Glu Asn Arg Cys Val Ala Leu Glu Arg C ys Pro Cys Phe His Gln
            820                 825                 830

Gly Gln Glu Tyr Ala Pro Gly Glu Thr Val L ys Ile Asp Cys Asn Thr
        835                 840                 845

Cys Val Cys Arg Asp Arg Lys Trp Thr Cys T hr Asp His Val Cys Asp
    850                 855                 860

Ala Thr Cys Ser Ala Ile Gly Met Ala His T yr Leu Thr Phe Asp Gly
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865                 870                 875                 880

Leu Lys Tyr Leu Phe Pro Gly Glu Cys Gln T yr Val Leu Val Gln Asp
                885                 890                 895

Tyr Cys Gly Ser Asn Pro Gly Thr Leu Arg I le Leu Val Gly Asn Glu
            900                 905                 910

Gly Cys Ser Tyr Pro Ser Val Lys Cys Lys L ys Arg Val Thr Ile Leu
        915                 920                 925

Val Glu Gly Gly Glu Ile Glu Leu Phe Asp G ly Glu Val Asn Val Lys
    930                 935                 940

Lys Pro Met Lys Asp Glu Thr His Phe Glu V al Val Glu Ser Gly Gln
945                 950                 955                 960

Tyr Val Ile Leu Leu Leu Gly Lys Ala Leu S er Val Val Trp Asp His
                965                 970                 975

Arg Leu Ser Ile Ser Val Thr Leu Lys Arg T hr Tyr Gln Glu Gln Val
            980                 985                 990

Cys Gly Leu Cys Gly Asn Phe Asp  Gly Ile Gln Asn Asn  Asp Phe Thr
        995                 1000                1005

Ser Ser  Ser Leu Gln Ile Glu  Glu Asp Pro Val Asp  Phe Gly Asn Ser
    1010                1015                1020

Trp  Lys Val Asn Pro Gln  Cys Ala Asp Thr Lys  Lys Val Pro Leu Asp
1025                1030                1035                1040

Ser Ser Pro Ala Val  Cys His Asn Asn Ile  Met Lys Gln Thr Met  Val
                1045                1050                1055

Asp Ser Ser Cys  Arg Ile Leu Thr Ser  Asp Ile Phe Gln Asp  Cys Asn
            1060                1065                1070

Arg Leu Val  Asp Pro Glu Pro Phe  Leu Asp Ile Cys Ile  Tyr Asp Thr
        1075                1080                1085

Cys Ser  Cys Glu Ser Ile Gly  Asp Cys Thr Cys Phe  Cys Asp Thr Ile
    1090                1095                1100

Ala  Ala Tyr Ala His Val  Cys Ala Gln His Gly  Lys Val Val Ala Trp
1105                1110                1115                1120

Arg Thr Ala Thr Phe  Cys Pro Gln Asn Cys  Glu Glu Arg Asn Leu  His
                1125                1130                1135

Glu Asn Gly Tyr  Glu Cys Glu Trp Arg  Tyr Asn Ser Cys Ala  Pro Ala
            1140                1145                1150

Cys Pro Ile  Thr Cys Gln His Pro  Glu Pro Leu Ala Cys  Pro Val Gln
        1155                1160                1165

Cys Val  Glu Gly Cys His Ala  His Cys Pro Pro Gly  Lys Ile Leu Asp
    1170                1175                1180

Glu  Leu Leu Gln Thr Cys  Ile Asp Pro Glu Asp  Cys Pro Val Cys Glu
1185                1190                1195                1200

Val Ala Gly Arg Arg  Leu Ala Pro Gly Lys  Lys Ile Ile Leu Asn  Pro
                1205                1210                1215

Ser Asp Pro Glu  His Cys Gln Ile Cys  Asn Cys Asp Gly Val  Asn Phe
            1220                1225                1230

Thr Cys Lys  Ala Cys Arg Glu Pro  Gly Ser Val Val Val  Pro Pro Thr
        1235                1240                1245

Asp Gly  Pro Ile Gly Ser Thr  Thr Ser Tyr Val Glu  Asp Thr Ser Glu
    1250                1255                1260

Pro  Pro Leu His Asp Phe  His Cys Ser Arg Leu  Leu Asp Leu Val Phe
1265                1270                1275                1280

Leu Leu Asp Gly Ser  Ser Lys Leu Ser Glu  Asp Glu Phe Glu Val  Leu
                1285                1290                1295
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Lys Val Phe Val  Val Gly Met Met Glu  His Leu His Ile Ser  Gln Lys
            1300                1305                1310

Arg Ile Arg  Val Ala Val Val Glu  Tyr His Asp Gly Ser  His Ala Tyr
        1315                1320                1325

Ile Glu  Leu Lys Asp Arg Lys  Arg Pro Ser Glu Leu  Arg Arg Ile Thr
    1330                1335                1340

Ser  Gln Val Lys Tyr Ala  Gly Ser Glu Val Ala  Ser Thr Ser Glu Val
1345                1350                1355                1360

Leu Lys Tyr Thr Leu  Phe Gln Ile Phe Gly  Lys Ile Asp Arg Pro  Glu
                1365                1370                1375

Ala Ser Arg Ile  Ala Leu Leu Leu Met  Ala Ser Gln Glu Pro  Ser Arg
            1380                1385                1390

Leu Ala Arg  Asn Leu Val Arg Tyr  Val Gln Gly Leu Lys  Lys Lys Lys
        1395                1400                1405

Val Ile  Val Ile Pro Val Gly  Ile Gly Pro His Ala  Ser Leu Lys Gln
    1410                1415                1420

Ile  His Leu Ile Glu Lys  Gln Ala Pro Glu Asn  Lys Ala Phe Val Phe
1425                1430                1435                1440

Ser Gly Val Asp Glu  Leu Glu Gln Arg Arg  Asp Glu Ile Ile Asn  Tyr
                1445                1450                1455

Leu Cys Asp Leu  Ala Pro Glu Ala Pro  Ala Pro Thr Gln His  Pro Pro
            1460                1465                1470

Met Ala Gln  Val Thr Val Gly Ser  Glu Leu Leu Gly Val  Ser Ser Pro
        1475                1480                1485

Gly Pro  Lys Arg Asn Ser Met  Val Leu Asp Val Val  Phe Val Leu Glu
    1490                1495                1500

Gly  Ser Asp Lys Ile Gly  Glu Ala Asn Phe Asn  Lys Ser Arg Glu Phe
1505                1510                1515                1520

Met Glu Glu Val Ile  Gln Arg Met Asp Val  Gly Gln Asp Arg Ile  His
                1525                1530                1535

Val Thr Val Leu  Gln Tyr Ser Tyr Met  Val Thr Val Glu Tyr  Thr Phe
            1540                1545                1550

Ser Glu Ala  Gln Ser Lys Gly Glu  Val Leu Gln Gln Val  Arg Asp Ile
        1555                1560                1565

Arg Tyr  Arg Gly Gly Asn Arg  Thr Asn Thr Gly Leu  Ala Leu Gln Tyr
    1570                1575                1580

Leu  Ser Glu His Ser Phe  Ser Val Ser Gln Gly  Asp Arg Glu Gln Val
1585                1590                1595                1600

Pro Asn Leu Val Tyr  Met Val Thr Gly Asn  Pro Ala Ser Asp Glu  Ile
                1605                1610                1615

Lys Arg Met Pro  Gly Asp Ile Gln Val  Val Pro Ile Gly Val  Gly Pro
            1620                1625                1630

His Ala Asn  Val Gln Glu Leu Glu  Lys Ile Gly Trp Pro  Asn Ala Pro
        1635                1640                1645

Ile Leu  Ile His Asp Phe Glu  Met Leu Pro Arg Glu  Ala Pro Asp Leu
    1650                1655                1660

Val  Leu Gln Arg Cys Cys  Ser Gly Glu Gly Leu  Gln Ile Pro Thr Leu
1665                1670                1675                1680

Ser Pro Thr Pro Asp  Cys Ser Gln Pro Leu  Asp Val Val Leu Leu  Leu
                1685                1690                1695

Asp Gly Ser Ser  Ser Ile Pro Ala Ser  Tyr Phe Asp Glu Met  Lys Ser
            1700                1705                1710
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Phe Thr Lys  Ala Phe Ile Ser Arg  Ala Asn Ile Gly Pro  Arg Leu Thr
        1715                1720                1725

Gln Val  Ser Val Leu Gln Tyr  Gly Ser Ile Thr Thr  Ile Asp Val Pro
    1730                1735                1740

Trp  Asn Val Ala Tyr Glu  Lys Val His Leu Leu  Ser Leu Val Asp Leu
1745                1750                1755                1760

Met Gln Gln Glu Gly  Gly Pro Ser Glu Ile  Gly Asp Ala Leu Ser  Phe
                1765                1770                1775

Ala Val Arg Tyr  Val Thr Ser Glu Val  His Gly Ala Arg Pro  Gly Ala
            1780                1785                1790

Ser Lys Ala  Val Val Ile Leu Val  Thr Asp Val Ser Val  Asp Ser Val
        1795                1800                1805

Asp Ala  Ala Ala Glu Ala Ala  Arg Ser Asn Arg Val  Thr Val Phe Pro
    1810                1815                1820

Ile  Gly Ile Gly Asp Arg  Tyr Ser Glu Ala Gln  Leu Ser Ser Leu Ala
1825                1830                1835                1840

Gly Pro Lys Ala Gly  Ser Asn Met Val Arg  Leu Gln Arg Ile Glu  Asp
                1845                1850                1855

Leu Pro Thr Val  Ala Thr Leu Gly Asn  Ser Phe Phe His Lys  Leu Cys
            1860                1865                1870

Ser Gly Phe  Asp Arg Val Cys Val  Asp Glu Asp Gly Asn  Glu Lys Arg
        1875                1880                1885

Pro Gly  Asp Val Trp Thr Leu  Pro Asp Gln Cys His  Thr Val Thr Cys
    1890                1895                1900

Leu  Pro Asp Gly Gln Thr  Leu Leu Lys Ser His  Arg Val Asn Cys Asp
1905                1910                1915                1920

Arg Gly Pro Arg Pro  Ser Cys Pro Asn Gly  Gln Pro Pro Leu Arg  Val
                1925                1930                1935

Glu Glu Thr Cys  Gly Cys Arg Trp Thr  Cys Pro Cys Val Cys  Met Gly
            1940                1945                1950

Ser Ser Thr  Arg His Ile Val Thr  Phe Asp Gly Gln Asn  Phe Lys Leu
        1955                1960                1965

Thr Gly  Ser Cys Ser Tyr Val  Leu Phe Gln Asn Lys  Glu Gln Asp Leu
    1970                1975                1980

Glu  Val Ile Leu Gln Asn  Gly Ala Cys Ser Pro  Gly Ala Lys Glu Thr
1985                1990                1995                2000

Cys Met Lys Ser Ile  Glu Val Lys His Asp  Gly Leu Ser Val Glu  Leu
                2005                2010                2015

His Ser Asp Met  Gln Met Thr Val Asn  Gly Arg Leu Val Ser  Ile Pro
            2020                2025                2030

Tyr Val Gly  Gly Asp Met Glu Val  Asn Val Tyr Gly Thr  Ile Met Tyr
        2035                2040                2045

Glu Val  Arg Phe Asn His Leu  Gly His Ile Phe Thr  Phe Thr Pro Gln
    2050                2055                2060

Asn  Asn Glu Phe Gln Leu  Gln Leu Ser Pro Arg  Thr Phe Ala Ser Lys
2065                2070                2075                2080

Thr Tyr Gly Leu Cys  Gly Ile Cys Asp Glu  Asn Gly Ala Asn Asp  Phe
                2085                2090                2095

Ile Leu Arg Asp  Gly Thr Val Thr Thr  Asp Trp Lys Ala Leu  Ile Gln
            2100                2105                2110

Glu Trp Thr  Val Gln Gln Leu Gly  Lys Thr Ser Gln Pro  Val His Glu
        2115                2120                2125

Glu Gln  Cys Pro Val Ser Glu  Phe Phe His Cys Gln  Val Leu Leu Ser
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    2130                2135                2140

Glu  Leu Phe Ala Glu Cys  His Lys Val Leu Ala  Pro Ala Thr Phe Tyr
2145                2150                2155                2160

Ala Met Cys Gln Pro  Asp Ser Cys His Pro  Lys Lys Val Cys Glu  Ala
                2165                2170                2175

Ile Ala Leu Tyr  Ala His Leu Cys Arg  Thr Lys Gly Val Cys  Val Asp
            2180                2185                2190

Trp Arg Arg  Ala Asn Phe Cys Ala  Met Ser Cys Pro Pro  Ser Leu Val
        2195                2200                2205

Tyr Asn  His Cys Glu His Gly  Cys Pro Arg Leu Cys  Glu Gly Asn Thr
    2210                2215                2220

Ser  Ser Cys Gly Asp Gln  Pro Ser Glu Gly Cys  Phe Cys Pro Pro Asn
2225                2230                2235                2240

Gln Val Met Leu Glu  Gly Ser Cys Val Pro  Glu Glu Ala Cys Thr  Gln
                2245                2250                2255

Cys Ile Ser Glu  Asp Gly Val Arg His  Gln Phe Leu Glu Thr  Trp Val
            2260                2265                2270

Pro Ala His  Gln Pro Cys Gln Ile  Cys Thr Cys Leu Ser  Gly Arg Lys
        2275                2280                2285

Val Asn  Cys Thr Leu Gln Pro  Cys Pro Thr Ala Lys  Ala Pro Thr Cys
    2290                2295                2300

Gly  Pro Cys Glu Val Ala  Arg Leu Arg Gln Asn  Ala Val Gln Cys Cys
2305                2310                2315                2320

Pro Glu Tyr Glu Cys  Val Cys Asp Leu Val  Ser Cys Asp Leu Pro  Pro
                2325                2330                2335

Val Pro Pro Cys  Glu Asp Gly Leu Gln  Met Thr Leu Thr Asn  Pro Gly
            2340                2345                2350

Glu Cys Arg  Pro Asn Phe Thr Cys  Ala Cys Arg Lys Asp  Glu Cys Arg
        2355                2360                2365

Arg Glu  Ser Pro Pro Ser Cys  Pro Pro His Arg Thr  Pro Ala Leu Arg
    2370                2375                2380

Lys  Thr Gln Cys Cys Asp  Glu Tyr Glu Cys Ala  Cys Asn Cys Val Asn
2385                2390                2395                2400

Ser Thr Val Ser Cys  Pro Leu Gly Tyr Leu  Ala Ser Ala Val Thr  Asn
                2405                2410                2415

Asp Cys Gly Cys  Thr Thr Thr Thr Cys  Phe Pro Asp Lys Val  Cys Val
            2420                2425                2430

His Arg Gly  Thr Ile Tyr Pro Val  Gly Gln Phe Trp Glu  Glu Ala Cys
        2435                2440                2445

Asp Val  Cys Thr Cys Thr Asp  Leu Glu Asp Ser Val  Met Gly Leu Arg
    2450                2455                2460

Val  Ala Gln Cys Ser Gln  Lys Pro Cys Glu Asp  Asn Cys Leu Ser Gly
2465                2470                2475                2480

Phe Thr Tyr Val Leu  His Glu Gly Glu Cys  Cys Gly Arg Cys Leu  Pro
                2485                2490                2495

Ser Ala Cys Glu  Val Val Thr Gly Ser  Pro Arg Gly Asp Ala  Gln Ser
            2500                2505                2510

His Trp Lys  Asn Val Gly Ser His  Trp Ala Ser Pro Asp  Asn Pro Cys
        2515                2520                2525

Leu Ile  Asn Glu Cys Val Arg  Val Lys Glu Glu Val  Phe Val Gln Gln
    2530                2535                2540

Arg  Asn Val Ser Cys Pro  Gln Leu Asn Val Pro  Thr Cys Pro Thr Gly
2545                2550                2555                2560
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Phe Gln Leu Ser Cys  Lys Thr Ser Glu Cys  Cys Pro Thr Cys His  Cys
                2565                2570                2575

Glu Pro Leu Glu  Ala Cys Leu Leu Asn  Gly Thr Ile Ile Gly  Pro Gly
            2580                2585                2590

Lys Ser Leu  Met Ile Asp Val Cys  Thr Thr Cys Arg Cys  Thr Val Pro
        2595                2600                2605

Val Gly  Val Ile Ser Gly Phe  Lys Leu Glu Gly Arg  Lys Thr Thr Cys
    2610                2615                2620

Glu  Ala Cys Pro Leu Gly  Tyr Lys Glu Glu Lys  Asn Gln Gly Glu Cys
2625                2630                2635                2640

Cys Gly Arg Cys Leu  Pro Ile Ala Cys Thr  Ile Gln Leu Arg Gly  Gly
                2645                2650                2655

Gln Ile Met Thr  Leu Lys Arg Asp Glu  Thr Ile Gln Asp Gly  Cys Asp
            2660                2665                2670

Ser His Phe  Cys Lys Val Asn Glu  Arg Gly Glu Tyr Ile  Trp Glu Lys
        2675                2680                2685

Arg Val  Thr Gly Cys Pro Pro  Phe Asp Glu His Lys  Cys Leu Ala Glu
    2690                2695                2700

Gly  Gly Lys Ile Met Lys  Ile Pro Gly Thr Cys  Cys Asp Thr Cys Glu
2705                2710                2715                2720

Glu Pro Glu Cys Lys  Asp Ile Ile Ala Lys  Leu Gln Arg Val Lys  Val
                2725                2730                2735

Gly Asp Cys Lys  Ser Glu Glu Glu Val  Asp Ile His Tyr Cys  Glu Gly
            2740                2745                2750

Lys Cys Ala  Ser Lys Ala Val Tyr  Ser Ile His Met Glu  Asp Val Gln
        2755                2760                2765

Asp Gln  Cys Ser Cys Cys Ser  Pro Thr Gln Thr Glu  Pro Met Gln Val
    2770                2775                2780

Ala  Leu Arg Cys Thr Asn  Gly Ser Leu Ile Tyr  His Glu Ile Leu Asn
2785                2790                2795                2800

Ala Ile Glu Cys Arg  Cys Ser Pro Arg Lys  Cys Ser Lys
                2805                2810
```

<210> SEQ ID NO 3
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Canine

<400> SEQUENCE: 3

aggggtttc caaaatgaca aaagagtgag cctctccgtg tatctcggag a atttttcga 60

<210> SEQ ID NO 4
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Canine

<400> SEQUENCE: 4

cattcatttg tttgtcaatg gtaccatgct gcaggggacc caaaggtaag t cagaagccc 60

<210> SEQ ID NO 5
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Canine

<400> SEQUENCE: 5

gaatgttcag gttaatatgg accctgggga tcactttgca acccccttgt t ttttcagat 60

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<210> SEQ ID NO 6
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Canine

<400> SEQUENCE: 6

gagggagccg gggcccagag acaggaagta aatgtgccca gggaaagtga g tggcaggac 60

<210> SEQ ID NO 7
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Canine

<400> SEQUENCE: 7

tgggtgaaag ccccatatcc cgactcctgg tcaaggagac tttgcaccaa g gtcccagcc 60

<210> SEQ ID NO 8
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Canine

<400> SEQUENCE: 8

ctggagcatg gggttggggt tggaaggtgg agggacatgg aggaaatgca t gagaagcac 60

<210> SEQ ID NO 9
<211> LENGTH: 58
<212> TYPE: DNA
<213> ORGANISM: Canine

<400> SEQUENCE: 9

gcttcctgag ctcctccttg tcccaccagc atctccatgc cctacgccta c aatgggc 58

<210> SEQ ID NO 10
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Canine

<400> SEQUENCE: 10

aaatgacaaa agagtgagcc ggtc 24

<210> SEQ ID NO 11
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Canine

<400> SEQUENCE: 11

aagtctcctt gaccagcggt cggg 24

<210> SEQ ID NO 12
<211> LENGTH: 35
<212> TYPE: PRT
<213> ORGANISM: Canine

<400> SEQUENCE: 12

Gly Gly Phe Gln Asn Asp Lys Arg Val Ser L eu Ser Val Tyr Leu Gly
1 5 10 15

Glu Phe Phe Asp Ile His Leu Phe Val Asn G ly Thr Met Leu Gln Gly
20 25 30

Thr Gln Arg
35

<210> SEQ ID NO 13

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<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Canine

<400> SEQUENCE: 13

Ile Ser Met Phe Tyr Ala Ser Asn Gly
1 5

We claim:

1. An isolated nucleic acid comprising a nucleotide sequence encoding the polypeptide of SEQ ID NO. 2, having a mutation at codon 85.

2. The isolated nucleic acid of claim 1, wherein the mutation is a deletion.

3. A vector comprising the nucleic acid of claim 1.

4. A vector comprising the nucleic acid of claim 2.

5. A cell comprising the vector of claim 3.

6. A cell comprising the vector of claim 4.

7. The isolated nucleic acid of claim 1, wherein the nucleotide sequence is capable of hybridizing under high stringency conditions to the complement of SEQ ID NO. 1 having a base deletion at codon 85.

8. A vector comprising the nucleic acid of claim 7.

9. A cell comprising the vector of claim 8.

10. A method of detecting a canine von Willebrand Factor gene in a sample comprising the steps of:

a) contacting the sample with an oligonucleotide comprising contiguous nucleotides of the nucleic acid sequence of SEQ ID NO. 1 having a base deletion at codon 85, and capable of specifically hybridizing with the canine von Willebrand Factor gene, under conditions favorable for hybridization of the oligonucleotide to any complementary sequences of nucleic acid in the sample; and

b) detecting hybridization, thereby detecting a canine von Willebrand Factor gene.

11. The method of claim 10, further comprising the step of:

c) quantifying hybridization of the oligonucleotide to complementary sequence.

12. A method of detecting a canine von Willebrand Factor gene in a sample comprising the steps of:

a) contacting the sample with an oligonucleotide comprising contiguous nucleotides of the nucleic acid sequence that is complementary to the sequence of SEQ ID NO. 1 having a base deletion at codon 85, and capable of specifically hybridizing to the complementary nucleotide sequence, under conditions favorable for hybridization of the oligonucleotide to any complementary sequences of nucleic acid in the sample; and

b) detecting hybridization, thereby detecting a canine von Willebrand Factor gene.

13. The method of claim 12, further comprising the step of:

c) quantifying ybidization of the oligonucleotide to complementary sequences.

14. An assay kit for screening for a canine von Willebrand Factor gene comprising:

a) an oligonucleotide comprising contiguous nucleotides from the nucleic acid sequence that is complementary to the sequence of SEQ ID NO. 1 having a base deletion at codon 85, and capable of specifically hybridizing to the complementary nucleotide sequence; and

b) reagents for hybridization of the oligonucleotide to a complementary nucleic acid sequence.

15. An oligonucleotide probe capable of detecting a mutation associated with canine von Willebrand's disease, wherein the mutation is a base deletion at codon 85 of the canine von Willebrand Factor gene.

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