CHAPTER IV

RESULTS

I. Identification of polymorphic sites in the cDNA sequence of the porcine vinculin gene.

Nine pairs of primers as shown in Table 1 were derived from the cDNA sequence of the porcine vinculin gene (Ponsuksili *et al.*, 1999 ; Accession number AF165172) suitable to amplify nine overlapping fragments covering the whole sequence of 5172 base pair (bp) with 41 bp of 5′ untranslated regions, 3203 bp translated sequence and 1928 bp of 3′ untranslated region. The position of the primers, start and stop codon are illustrated in Figure 16. The start codon (ATG) is in fragment 1 and the stop codon (TAG) is in fragment 6.

The porcine sequence has 94% homology to the human cDNA sequence. The amino acid residues show 99% identities. The human protein consists of 1066 amino acids whereas the deducted porcine protein has 1067 amino acid residues, with one additional lysine at position 262. Comparison of amino acid sequence of the porcine and human vinculin is given in Figure 17.

To detect polymorphisms in the vinculin gene the nine overlapping fragments were amplified with the primer set mentioned above from animals of five different breeds, cloned and comparatively sequenced. The sequences of the cDNA fragments

were compared using the BLAST software (http://www.ncbi.nlm.nih. gov/) and multiple sequences aligment on the World Wild Web site of BCM (http://dot.imgen.bcm.tmc.edu). First analysis of the sequences revealed 7 potential single nucleotide polymorpisms, SNPs, at the positions 21 (A to C in F2), 213 (G to A in Duroc), 294 (C to T in Hamshire), 1575 (C to T in F2 and Hamshire), 2382 (C to A in Hamshire), 2457 (A to G in Hamshire) and 4197 (G to T in German Landrace) (showed in table 2), the first four NSPs four clones were selected per animal and another NSPs three clones were selected.

In order to confirm the polymorphisms each fragment was repeatedly sequenced that revealed a potential polymorphic site after the first analysis. Therefore, all steps beginning with the PCR were repeated from the animal suspicious to carry a new allele and one of the other animals. For each of the fragments three clones were selected per animal and comparatively sequenced. Three of the SNPs at the positions 2382, 2457 and 4197 (figure 18-20) were confirmed. The comparision of amino acid sequences derived for the sequences revealed that the polymorphisms do not affect the amino acid sequence.

II. Establishment of protocols for PCR-based genotypic at the polymorphic sites of the vinculin gene.

In order to derive PCR-RFLPs to allow genotyping at the three polymorphic sites the solftware package "webcutter" available at the World Wild Web site of BCM was used to screen for restriction enzyme suitable to differentiate between the allelic variants. It was found that the restrictions enzymes XcmI, BfaI and MboI will be allowed to genotype at the positions 2382, 2457 and 4197 respectively. Therefore, new primers

were derived that lead to the production of fragments of 146 bp, 292 bp and 371 bp, repectively, from genomic DNA. For the polymorphic site at position 2382 the two different alleles that were obtained reveal fragments of 146bp (uncut) or 94 and 52 bp (cut). The PCR-RFLP used to genotype the SNP at position 2457 gives one bands representing a fragment of 292 bp for one allele and two fragments of 233 and 59 bp for the other allele. The 371 bp product of the PCR designed to genotype the SNP at position 4197 has a constant cutting site for MboI. Therefore, one allele is represented by fragments of 203 and 168 bp and the other allele is represented by three fragments of 203, 115 and 53 bp length in the PCR-RFLP.

III. Physical mapping of the porcine vinculin gene

The use ot the nine primer pairs which had been successfully used for amplification starting with cDNA, to amplify the corresponding regions starting with genomic DNA revealed detectable bands only for fragments vin 6, 7, 8, 9. These primer pairs were tested for their suitability to be applied for chromosomal assignment using the ImpRH panel. Since primer pair vin 8 up/dw revealed a clear strong band from porcine genomic DNA but not from hamster DNA, it was chosen for physical mapping. Regional assignment was achieved through the concordant segregation of PCR products and chromosome fragments retained in the hybrid cells (Robic *et al.*, 1996). Statistical analyses were achieved using the computer program which is accessible on the World Wide Web site of INRA. Vinculin was assigned to chromosome 14 in 25 cR and 45 cR distance to microsatellites SW1536 and SW2105 (LOD score 13.89 and 8.92).

Table 1 Primers for amplified porcine vinculin gene.

Primers	Oligonucleotide Sequences (5'- 3')	Annealing Temperature(°C)
Vin1fw	TTCTCTGTAGCCCGCGGTTC	61
Vin1rev	CGTCAATCATCTTGGCCATC	
Vin2fw	AAGATGATTGACGAGAGGCAG	57
Vin2rev	ATGCTCTGCTTTGAGTTGGTC	
Vin3fw	GAAGCCATGACCAACTCA	59
Vin3rev	CTGTGCTTGCTCAATCTTGC	
Vin4fw	GCAAGATTGAGCAAGCACAG	61
Vin4rev	AACCTGGGGTGTGAGTTCTC	
Vin5fw	TCACACCCCAGGTTGTCTCAG	61
Vin5rev	ACCTCACCCTCAGGCAGAGG	
Vin6fw	CTGACAGATGAGCTTGCTCCTC	60-57 (TD)
Vin6rev	CTTTCCATGTTGCTGCTGACTC	
Vin7fw	CTACCTGGTTCCTTTTCAGAAG	58
Vin7rev	CTCATTTAGGAAGGATCTGAGG	
Vin8fw	TTCTTTCTGAGCTGAAATGCTG	63-58 (TD)
Vin8rev	GAATTCTTGCCTGAATGTCCTT	
Vin9fw	AACTATGTTTCTTCCCAAAGC	54
Vin9rev	AGAAGAGGCGGAAAATATTTG	

Figure 16 Porcine vinculin sequence, showing primer sequences, start and stop codon and point mutation positions.

LOCUS /tmp/readseq.in.28663 5172 bp

DEFINITION /tmp/readseq.in.28663 [Unknown form], 5172 bases, DE2 checksum.

ORIGIN

Position 1

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   1  TTCTCTGTAG CCCGCGGTTC GCCGCCCCGC TAGCCGCCGC GATGCCGGTG
                 21
  51  TTTCATACGC GAACGATCGA GAGCATCCTG GAGCCGGTGG CGCAGCAGAT
 101  CTCGCACCTG GTGATCATGC ACGAGGAAGG CGAGGTGGAC GGCAAAGCCA
 151  TCCCTGACCT CACCGCGCCT GTGGCCGCCG TGCAGGCGCG GTCAGCAACC
 201  TCGTCCGGGT TGGAAAAGAG ACTGTTCAAA CCACTGAGGA TCAGATTTTG
       213
 251  AAGAGAGATA TGCCACCAGC ATTTATTAAG GTTGAGAATG CTTGTACCAA
                                      294
 301  GCTTGTCCAG GCAGCCCAGA TGCTTCAGTC AGACCCTTAC TCAGTGCCTG
 351  CTCGAGACTA TCTGATCGAT GGGTCAAGGG GCATCCTCTC CGGCACATCA
 401  GACCTGCTCC TCACCTTCGA TGAGGCTGAG GTTCGTAAAA TTATTAGAGT
 451  TTGCAAAGGA ATTTTGGAAT ATCTTACAGT GGCAGAAGTG GTGGAAACTA
 501  TGGAAGATTT GGTCACTTAC ACAAAGAATC TTGGACCAGG AATGACTAAG
 551  ATGGCCAAGA TGATTGACGA GAGGCAGCAG GAACTGACTC ACCAGGAGCA
 601  CCGAGTGATG TTGGTGAATT CAATGAACAC TGTAAAAGAG CTGTTGCCAG
 651  TTCTCATTTC CGCTATGAAG ATTTTTGTAA CAACTAAAAA CTCAAAAAAC
 701  CAAGGAATAG AAGAAGCTTT GAAAAATCGC AATTTTACTG TAGAAAAGAT
 751  GAGTGCAGAA ATTAATGAAA TCATTCGTGT ATTACAACTC ACTTCTTGGG
 801  ACGAAGATGC CTGGGCCAGC AAGAAGGACA CTGAAGCCAT GAAGAGAGCC
 851  TTGGCTTCCA TAGACTCCAA ACTGAACCAG GCCAAAGGTT GGCTTCGTGA
 901  CCCCACTGCC TCCCCAGGTG ATGCTGGTGA GCAGGCCATC AGGCAGATCT
 951  TAGATGAAGC TGGAAAAGTC GGTGAACTCT GTGCAGGCAA AGAACGCAGG
1001  GAGATCCTGG GAACCTGCAA AATGCTAGGG CAGATGACTG ATCAAGTGGC
1051  TGACCTCCGA GCCAGAGGAC AAGGAGCCTC ACCGGTGGCC ATGCAGAAAG
1101  CCCAGCAGGT GTCTCAGGGT CTGGATGTGC TCACAGCTAA AGTGGAAAAT
1151  GCAGCCCGCA AGCTGGAAGC CATGACCAAC TCAAAGCAGA GCATTGCGAA
1201  GAAGATCGAT GCTGCTCAGA ATTGGCTCGC AGATCCAAAT GGTGGACCAG
1251  AAGGAGAAGA ACAGATTCGA GGGGCCTTGG CTGAAGCTCG GAAAATAGCA
1301  GAATTATGTG ATGATCCTAA AGAGAGAGAT GACATTCTGC GTTCTCTTGG
1351  AGAAATATCT GCTCTGACTT CTAAATTAGC AGATCTACGA AGACAGGGAA
1401  AAGGAGATTC CCCAGAGGCC CGCGCATTGG CCAAACAGGT GGCCACGGCC
1451  CTGCAGAACT TGCAGACCAA AACCAACAGG GCTGTGGCCA ACAGCAGACC
1501  TGCCAAAGCA GCTGTGCACC TTGAGGGCAA GATTGAGCAA GCACAGCGGT
1551  GGATTGATAA TCCCACAGTG GATGACCGGG GAGTTGGTCA GGCTGCCATC
                 1575
1601  CGGGGGCTTG TAGCCGAAGG GCATCGTCTG GCTAATGTCA TGATGGGGCC
1651  TTATCGCCAA GATCTTCTGG CAAAGTGTGA CCGAGTGGAC CAGCTGACAG
1701  CCCAGCTGGC TGACCTGGCT GCCAGAGGGG AAGGGGAGAG TCCTCAGGCG
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1751  AGAGCACTTG CATCTCAACT CCAAGACTCC TTAAAGGATC TGAAAGCCCG
1801  GATGCAAGAA GCCATGACTC AAGAGGTGTC AGATGTTTTC AGTGATACCA
1851  CAACTCCCAT CAAGCTGCTG GCAGTGGCAG CCACTGCCCC TCCTGATGCA
1901  CCCAATAGAG AAGAGGTGTT TGATGAGAGG GCAGCTAACT TTGAAAACCA
1951  TTCAGGAAGG CTTGGTGCCA CAGCAGAGAA GGCGGCTGCA GTTGGAACTG
2001  CTAATAAATC AACAGTAGAA GGCATTCAGG CCTCAGTGAA GACAGCCCGA
2051  GAACTCACAC CCCAGGTTGT CTCAGCCGCT CGCATCTTAC TTAGGAATCC
2101  TGGAAATCAA GCTGCTTATG AACATTTTGA GACCATGAAG AACCAGTGGA
2151  TTGATAATGT TGAAAAAATG ACAGGGTTGG TGGACGAAGC CATTGACACC
2201  AAATCTCTGT TGGATGCTTC CGAAGAAGCG ATTAAAAAAG ACCTGGACAA
2251  GTGTAAAGTA GCCATGGCCA ACATTCAGCC TCAGATGCTG GTTGCTGGGG
2301  CAACCAGCAT CGCTCGTCGG GCCAACCGCA TTCTGCTGGT GGCTAAGAGG
2351  GAGGTGGAGA ACTCTGAGGA TCCCAAGTTC CGTGAGGCGG TGAAAGCTGC
                          2382
2401  CTCTGACGAA TTGAGCAAAA CCATCTCCCC AATGGTGATG GATGCAAAGG
                                                    2457
2451  CTGTGGCAGG AAACATTTCT GACCCTGGCC TGCAAAAGAG CTTCCTAGAC
2501  TCAGGATACC GGATCCTGGG AGCCGTGGCC AAGGTGAGAG AAGCCTTCCA
2551  ACCTCAGGAG CCTGACTTCC CGCCTCCTCC GCCAGACCTT GAACAGCTCC
2601  GCCTGACAGA TGAGCTTGCT CCTCCCAAAC CACCTCTGCC TGAGGGTGAG
2651  GTCCCTCCGC CCAGGCCTCC ACCACCAGAG GAGAAGGATG AAGAGTTCCC
2701  TGAGCAGAAA GCCGGAGAGG TGATTAACCA GCCAATGATG ATGGCTGCCA
2751  GGCAGCTCCA TGATGAAGCT CGCAAATGGT CCAGTAAGGG CAATGACATC
2801  ATTGCAGCAG CCAAGCGCAT GGCTCTGCTA ATGGCCGAGA TGTCTCGGCT
2851  GGTCAGAGGG GGCAGTGGTA CCAAGCGGGC ACTGATTCAG TGTGCCAAGG
2901  ACATCGCCAA GGCCTCAGAC GAGGTGACTC GGTTGGCCAA GGAGGTTGCC
2951  AAGCAGTGCA CAGATAAGCG GATTAGAACC AACCTCTTAC AGGTATGCGA
3001  GCGAATCCCA ACCATAAGCA CCCAGCTTAA AATCCTGTCC ACAGTGAAGG
3051  CCACCATGCT GGGCCGGACC AACATCAGTG ATGAGGAGTC TGAGCAGGCC
3101  ACAGAGATGC TGGTTCACAA TGCCCAGAAT CTGATGCAAT CTGTGAAGGA
3151  GACTGTTCGA GAAGCAGAAG CAGCTTCAAT CAAAATTAGA ACGGATGCTG
3201  GATTTACACT GCGCTGGGTT AGAAAGACTC CCTGGTACCA GTAGACACCT
3251  GGTTGAACCT GGCTAACATA GAAACCCCTG CTAAACAGAA TGAAAATGGT
3301  CTGAGTCCCA GGAACTGCCC AGGATTGCTG GGGGGGTTGA AAGCCACATC
3351  CTGGCCTGAC CTATCAGAAA GGAATGGGGC CTCTTCATAT TAGAAAGCAT
3401  TTATACTCTT GTCTCGGACA CTTTGAAATG TGTCTCCGTA TAAAGCCTGT
3451  ATTCTCAAAC ACGGTTTCAC TCGTGCACAC TATCCCAGGA GGCAGACTGG
3501  GTTTCCAGCC CATGGACTTC ACATAAGCTC AGAATCCAAG ACTGAACACT
3551  AGCCAGACAC CCTGCTCTGC CCTTGTTCCC TTTCTGCTCC ACCCCTAGTT
3601  CTCGTCACCA GGCTCCCAAG ACCCATGGCC CAGGCATGTC GGTTAAGAAG
3651  GAAAAATCAC TGTGCCTCCA AAATTTGTCT TGGGCTTTCC ATGTTGCTGC
3701  TGACTCGCCT GCTTTCCCTG GCTGTGCTAC CTGGTTCCTT TTCAGAAGTG
3751  AGCTTCGCTG CTGTAGGGGA AAGTGGCTTC TGGGGAGCCC GGGCATATAG
3801  GGCCTGGATT CACTTCCTGC CCTTCCCCAA TTTAGTCCTT CTAGTCTCCC
3851  ACGATACAAA ACCAAATTTC ACTTGTCAGG AGGAAATCAC AGAATGGTGT
3901  GATTTTTGTC TTTACCTCAC CCCAGAGCAG TGTCTGTGCT AGGGAAACTT
3951  TCCGTCCCAT ATCCTGCCTC GGCCTGCCCA GGCAGCCATC CCAAGAATAC
4001  ACTGTGTGTC CTGGTGCTCT CTGCCACTGG AGAGGCAGAG TAGCCAGGGC
4051  GTGGCCCTGC CCATCCTCCC AGCAGGGACA CTCCCAGGCG CTCCACGCTT
4101  TGTCACTGCC TGCAGAGATC TGTGCTGAGG CCTTACCATT CATTCTTAGC
4151  TCTTATTGTT CTTTCTGAGC TGAAATGCTG CATTTAATTT TTAACCAAAT
                                      4197
4201  CATGTCTCCT GTCCTGGCTT TTGTATTCTT CCCTCAGATC CTTCCTAAAT
4251  GAGATTTTAA AGATATGTGT TTGTTCTATA ATTTTGAAAG ATCCTTTTTA
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4301  TCCTTTTGAA ATTTAACCCT AAGAATTGGT GCTTGTCCCC CCAGGAGTGT
4351  TTAATGGAAA GGCAATCCTG TTTGCAGGAC ACTTCCTACG TAAGGGAGGT
4401  GGTTACCTGC AGACTGGAAT TCTGGCACTG CTGGGGATAA ATCAATGGAA
4451  AGTAGTCCTC AGTAATTCCT CCCTCTCTCA GCCAACAACC ACCAAGCCCT
4501  GTGCCTCCTC CCCTCCCAAG TACAGTTATT CGAGAGACTA ATAGGTATAA
4551  TATTTAATTA TCACCATAAT GTTTCCTATT AGCAAAGTCA GAGAGAAGGC
4601  AATTTTTCCT TTCCCACACT TACCACTGCT GTCTAAGCAT TCCCCAGCAC
4651  ATGAAACTAT GTTTCTTCCC AAAGCCAGAA CTGGATGAGT AAAGGAGTAA
4701  GAATTCTTGC CTGAATGTCC TTCCTTCCCA CTTCCAATGT GTGTTAGATC
4751  CTAACAGCAA ATGTGTAAAA CTTGTCTTAA GTTGGTACTG TACACTCAGG
4801  CTTCCTCTGT TTCCTTTTAA CTGATGACTA TTTTCAAGGC CCTCAGCATC
4851  TTTGTATAAT TGCTTACCTG ATATAAATGC AATATTAATG CCTTTAAAGT
4901  ATGAATCTAT GCCAAAGATC ACCTTTTGTT TTACTAAAGA TTACTTAGAG
4951  GAAAAAAGAA AAATCATGTT TGCTCTCCAA GTTCTTCCAG TGTTTTGAGA
5001  CACTGGCTTA CACTTTACGC CAATGTGCTT TTCTCTAATA TAGTGCTCAA
5051  GACACAGTGA AGCAAATTAA AAAAGAAAAA AAAAAATCCC CGAATGCTGA
5101  TTAGCGACAT CACCACTAAA AAAACATTTA TAAGCTAGGA TTTGTTATAT
5151  GCAAATATTT TCCGCCTCTT CT
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Table 2 Sequence changes in porcine vinculin gene.

Position	Base change	Within breed	Amino acid
21	A → C	F2	Arg→Arg
213	G → A	Duroc	Lys→Lys
294	C → T	Hamshire	Asp→Asp
1575	C → T	Hamshire and F2	Ala→Ala
2382	C → A	Hamshire	Thr→Thr
2457	A → G	Hamshire	Leu→Leu
4197	G → T	German Landrace	Gln→Gln

Table 3 Intron structure in fragment 1, estimated by comparative to the other species of vinculin gene.

Position in porcine vinculin	Length of Intron (bp)	Related to species
183	100	Human
185	100	Mouse
279	369	Human and Mouse
539	260	Human and Mouse

Figure 17 Comparision of human and porcine vinculin structure.

Sequence 1 Human vinculin (P18206) **Length** 1066 (1 .. 1066)

Sequence 2 Porcine vinculin **Length** 1067 (1 .. 1067)

NOTE: The statistics (bitscore and expect value) is calculated based on the size of nrdatabase

Score = 1884 bits (4826), Expect = 0.0

Identities = 1063/1067 (99%), Positives= 1066/1067(99%), Gaps= 1/1067(0%)

```
Query: 1 MPVFHTRTIESILEPVAQQISHLVIMHEEGEVDGKAIPDLTAPVAAVQAAVSNLVRVGKE 60
         MPVFHTRTIESILEPVAQQISHLVIMHEEGEVDGKAIPDLTAPVAAVQAAVSNLVRVGKE
Sbjct: 1 MPVFHTRTIESILEPVAQQISHLVIMHEEGEVDGKAIPDLTAPVAAVQAAVSNLVRVGKE 60

Query: 61 TVQTTEDQILKRDMPPAFIKVENACTKLVQAAQMLQSDPYSVPARDYLIDGSRGILSGTS 120
          TVQTTEDQILKRDMPPAFIKVENACTKLVQAAQMLQSDPYSVPARDYLIDGSRGILSGTS
Sbjct: 61 TVQTTEDQILKRDMPPAFIKVENACTKLVQAAQMLQSDPYSVPARDYLIDGSRGILSGTS 120
                                                    168
                                                     ↓
Query:121 DLLLTFDEAEVRKIIRVCKGILEYLTVAEVVETMEDLVTYTKNLGPGMTKMAKMIDERQQ 180
          DLLLTFDEAEVRKIIRVCKGILEYLTVAEVVETMEDLVTYTKNLGPGMTKMAKMIDERQQ
Sbjct:121 DLLLTFDEAEVRKIIRVCKGILEYLTVAEVVETMEDLVTYTKNLGPGMTKMAKMIDERQQ 180
```


208
Query:181ELTHQEHRVMLVNSMNTVKELLPVLISAMKIFVTTKNSKNQGIEEALKNRNFTVEKMSAE 240
ELTHQEHRVMLVNSMNTVKELLPVLISAMKIFVTTKNSKNQGIEEALKNRNFTVEKMSAE
Sbjct:181ELTHQEHRVMLVNSMNTVKELLPVLISAMKIFVTTKNSKNQGIEEALKNRNFTVEKMSAE 240
259
repetitive region1
Query:241INEIIRVLQLTSWDEDAWASK-DTEAMKRALASIDSKLNQAKGWLRDPSASPGDAGEQAI 299
INEIIRVLQLTSWDEDAWASK DTEAMKRALASIDSKLNQAKGWLRDP+ASPGDAGEQAI
Sbjct:241INEIIRVLQLTSWDEDAWASKKDTEAMKRALASIDSKLNQAKGWLRDPTASPGDAGEQAI 300
Query:300RQILDEAGKVGELCAGKERREILGTCKMLGQMTDQVADLRARGQGSSPVAMQKAQQVSQG 359
RQILDEAGKVGELCAGKERREILGTCKMLGQMTDQVADLRARGQG+SPVAMQKAQQVSQG
Sbjct:301RQILDEAGKVGELCAGKERREILGTCKMLGQMTDQVADLRARGQGASPVAMQKAQQVSQG 360
369
repetitive region2
Query:360LDVLTAKVENAARKLEAMTNSKQSIAKKIDAAQNWLADPNGGPEGEEQIRGALAEARKIA 419
LDVLTAKVENAARKLEAMTNSKQSIAKKIDAAQNWLADPNGGPEGEEQIRGALAEARKIA
Sbjct:361LDVLTAKVENAARKLEAMTNSKQSIAKKIDAAQNWLADPNGGPEGEEQIRGALAEARKIA 420
Query:420ELCDDPKERDDILRSLGEISALTSKLADLRRQGKGDSPEARALAKQVATALQNLQTKTNR 479
ELCDDPKERDDILRSLGEISALTSKLADLRRQGKGDSPEARALAKQVATALQNLQTKTNR
Sbjct:421ELCDDPKERDDILRSLGEISALTSKLADLRRQGKGDSPEARALAKQVATALQNLQTKTNR 480
480
repetitive region3
Query:480AVANSRPAKAAVHLEGKIEQAQRWIDNPTVDDRGVGQAAIRGLVAEGHRLANVMMGPYRQ 539
AVANSRPAKAAVHLEGKIEQAQRWIDNPTVDDRGVGQAAIRGLVAEGHRLANVMMGPYRQ
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589
Tendem repeat
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DLLAKCDRV RGEGESPQARALASQLQDSLKDLKARMQEAMTQEVSDVF
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SDTTTPIKLLAVAATAPPDAPNREEVFDERAANFENHS STVE
Sbjct:601SDTTTPIKLLAVAATAPPDAPNREEVFDERAANFENHSGRLGATAEKAAAVGTANKSTVE 660
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GIQASVKTARELTPQVVSAARILLRNPGNQAAYEHFETMKNQWIDNVEKMTGLVDEAIDT
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Sbjct:721KSLLDASEEAIKKDLDKCKVAMANIQPQMLVAGATSIARRANRILLVAKREVENSEDPKF 780
791
837
Query:780REAVKAASDELSKTISPMVMDAKAVAGNISDPGLQKSFLDSGYRILGAVAKVREAXXXXX 839
REAVKAASDELSKTISPMVMDAKAVAGNISDPGLQKSFLDSGYRILGAVAKVREA
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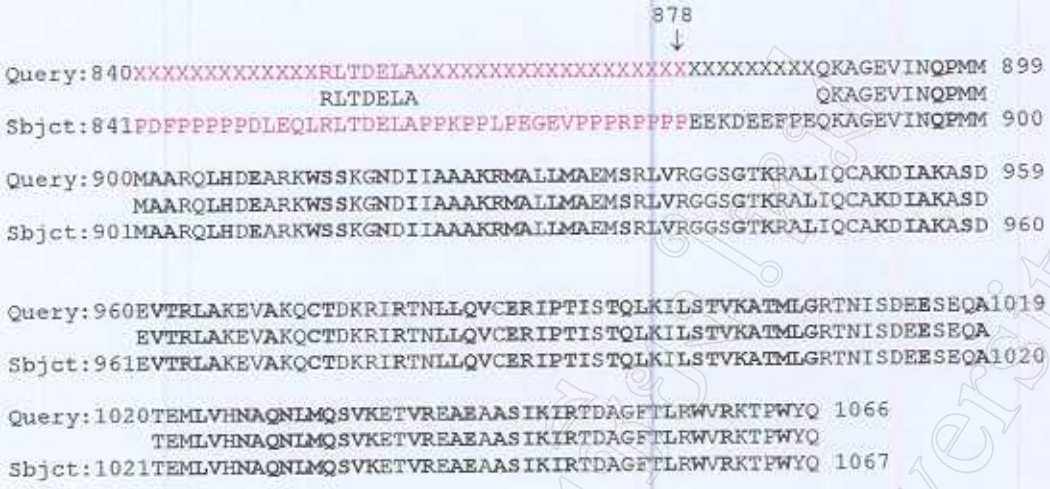

Figure 17 has shown the structure homology of human (M33308) and porcine vinculin amino acids sequences. Amino acids residue 1 – 258 is N-ternimal domain, central domain, 791-962 is C-terminal domain. Amino acid in bald letters are identical to *Drosophila* (X96601), *C.legans* (J04804), *Gallus gallus* (J04126) compared to porcine vinculin. These regions are highly conserved. The red letter at position 262 has shown the 1 amino acid inserted in Porcine vinculin but the other points are shown amino acid which encoded by single base change. The dark blue sequence shows the talin interaction region and the pink sequence shows the proline-rich region domain.

Figure 18 The point mutation at position 2382 with in Hamshire.

```
Hamshire          2382
      TTGAGCAAAACAATCTCCCCAATGGTGATGGATGCAAAGGCTGTGGCAGGAAACATTTC
Duroc
      TTGAGCAAAACCATCTCCCCAATGGTGATGGATGCAAAGGCTGTGGCAGGAAACATTTC
German Landrace
      TTGAGCAAAACCATCTCCCCAATGGTGATGGATGCAAAGGCTGTGGCAGGAAACATTTC
Pietrain
      TTGAGCAAAACCATCTCCCCAATGGTGATGGATGCAAAGGCTGTGGCAGGAAACATTTC
F2
      TTGAGCAAAACCATCTCCCCAATGGTGATGGATGCAAAGGCTGTGGCAGGAAACATTTC

consensus
      TTGAGCAAAACCATCTCCCCAATGGTGATGGATGCAAAGGCTGTGGCAGGAAACATTTC
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Figure 19 The point mutation at position 2457 within Hamshire (A to G , the picture show in N700 or anti-sense strand).

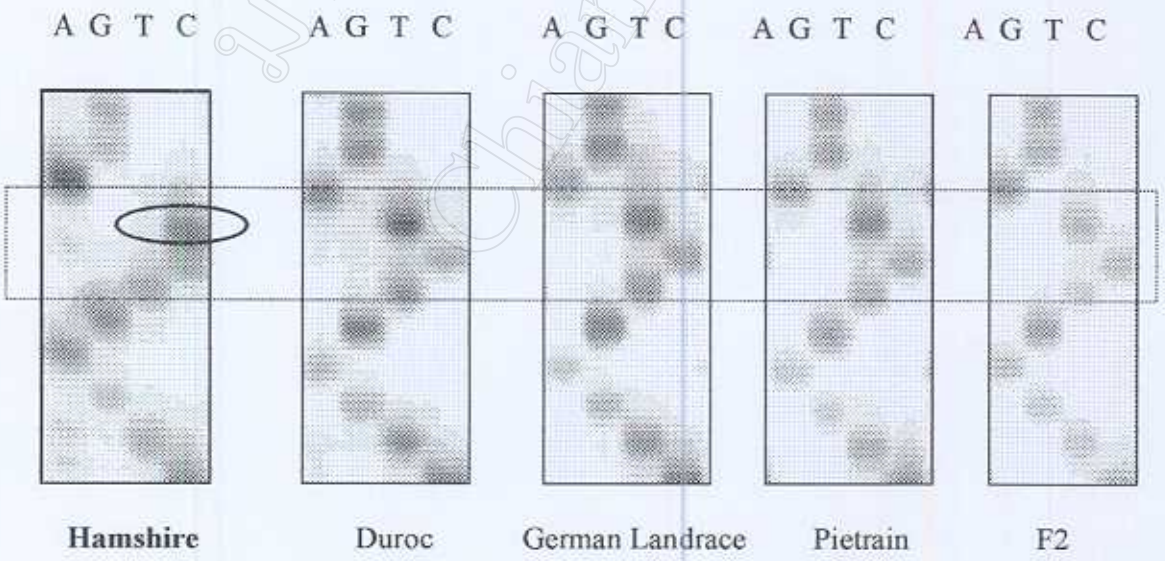

Figure 20 The point mutation at position 4197 within German Landrace (G to T).

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Hamshire
      TTTTGTATTCTTCCCTCAGATCCTTCCTAAATGAGATTTTAAAGATATGTGTTTGTTCTA
Duroc
      TTTTGTATTCTTCCCTCAGATCCTTCCTAAATGAGATTTTAAAGATATGTGTTTGTTCTA
German Landrace          4197
      TTTTGTATTCTTCCCTCATATCCTTCCTAAATGAGATTTTAAAGATATGTGTTTGTTCTA
Pietrain
      TTTTGTATTCTTCCCTCAGATCCTTCCTAAATGAGATTTTAAAGATATGTGTTTGTTCTA
F2
      TTTTGTATTCTTCCCTCAGATCCTTCCTAAATGAGATTTTAAAGATATGTGTTTGTTCTA

      ____________________________________________________________
consensus
      TTTTGTATTCTTCCCTCAGATCCTTCCTAAATGAGATTTTAAAGATATGTGTTTGTTCTA
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Figure 21 The sample to identify result of RH-panel.