Original Article

Cytogenet Genome Res 107:123–127 (2004)
DOI: 10.1159/000079582

Cytogenetic and Genome Research

Characterization and chromosome localization of a processed pseudogene related to the bovine laminin receptor gene family

M. Germerodt,[a] C. Knorr,[a] J. Beck,[a] C. Drögemüller,[b] J.L. Williams,[c] F. Habermann,[d] R. Fries[d] and B. Brenig[a]

[a] Institute of Veterinary Medicine, Georg August University, Göttingen;
[b] Institute of Animal Breeding and Genetics, School of Veterinary Medicine Hannover, Hannover (Germany);
[c] Roslin Institute (Edinburgh), Roslin, Midlothian (Scotland);
[d] Institute of Animal Breeding and Molecular Genetics, Technical University of Munich, Munich (Germany)

Abstract. A bovine BAC clone containing a processed laminin receptor pseudogene (LAMR1P) has been isolated and characterized. A 2,901-bp sequence was produced from the clone, of which 1,187 bp represented seven identifiable exon-like domains, but no intervening sequences. The pseudogene sequence reveals several transversions and transitions, as well as insertions and deletions. A premature stop codon motif is present at nucleotide position 115 located in the exon-2-like domain. Physical mapping of the gene was performed by FISH and RH panel mapping and assigned LAMR1P to BTA4q24→q26 with the closest linkage to BM6458 (19 cR, LOD score of 11.6). The functional laminin receptor putatively plays an important role in the transmission of bovine spongiform encephalopathy (BSE). In this process, the receptor supposedly acts as the binding site for prion proteins to enter mammalian cells. Considering the existence of several human laminin receptor pseudogenes forming a complex family, any knowledge of even pseudogene sequences might be helpful to isolate the functional bovine laminin receptor gene.

The 67-kDa laminin receptor (67 kDa LR) is a non-integrin cell surface receptor that mediates high-affinity interactions between mammalian cells and laminin, a glycoprotein of the extra-cellular matrix. After isolation and characterization of a cDNA clone representing the 67 kDa LR it became evident that the corresponding mRNA only contains a coding potential for a 32-kDa polypeptide. The translated protein finally turned out to possess a molecular mass of 37 kDa (Yow et al., 1988; Rao et al., 1989). The protein was called 37-kDa laminin receptor precursor (37 kDa LRP) assuming that the mature 67 kDa LR is assembled either by homodimerization of two 37 kDa LRP molecules or by heterodimerization of one 37 kDa LRP and a second so far unknown protein (Castronovo et al., 1991; Buto et al., 1998).

In humans, LR/LRP is involved in tumor progression and also acts as the major receptor for Sindbis (Wang et al., 1992) and tick-borne encephalitis virus (Protopopova et al., 1997). Recently yeast two-hybrid screening identified the 37 kDa LRP also as the putative receptor for the prion protein (PrPsc) (Rieger et al., 1997), the causative agent of transmissible spongiform encephalopathy (TSE) (Prusiner, 1982). Cell binding and internalization studies on neuronal and non-neuronal cells confirm the hypothesis that the 37 kDa LRP acts as the binding site enabling PrPc/PrPsc to invade mammalian cells. Moreover, although oral intake of PrPsc-contaminated fodder has been highlighted as the route of infection with bovine spongiform encephalopathy (BSE), still little is known about how the exogenous infectious agent enters the intestinal mucosa. The mecha-

Supported by a grant of the Erxleben Research and Innovation Council to B. Brenig (ERIC-BR1959-2001-4).

Received 15 March 2004; manuscript accepted 1 April 2004.

Request reprints from B. Brenig, Institute of Veterinary Medicine
Groner Landstrasse 2, DE–37073 Göttingen (Germany)
telephone: +49 551 393383; fax: +49 551 393392
e-mail: bbrenig@gwdg.de

0301–0171/04/1072–0123$21.00/0

Accessible online at:
www.karger.com/cgr

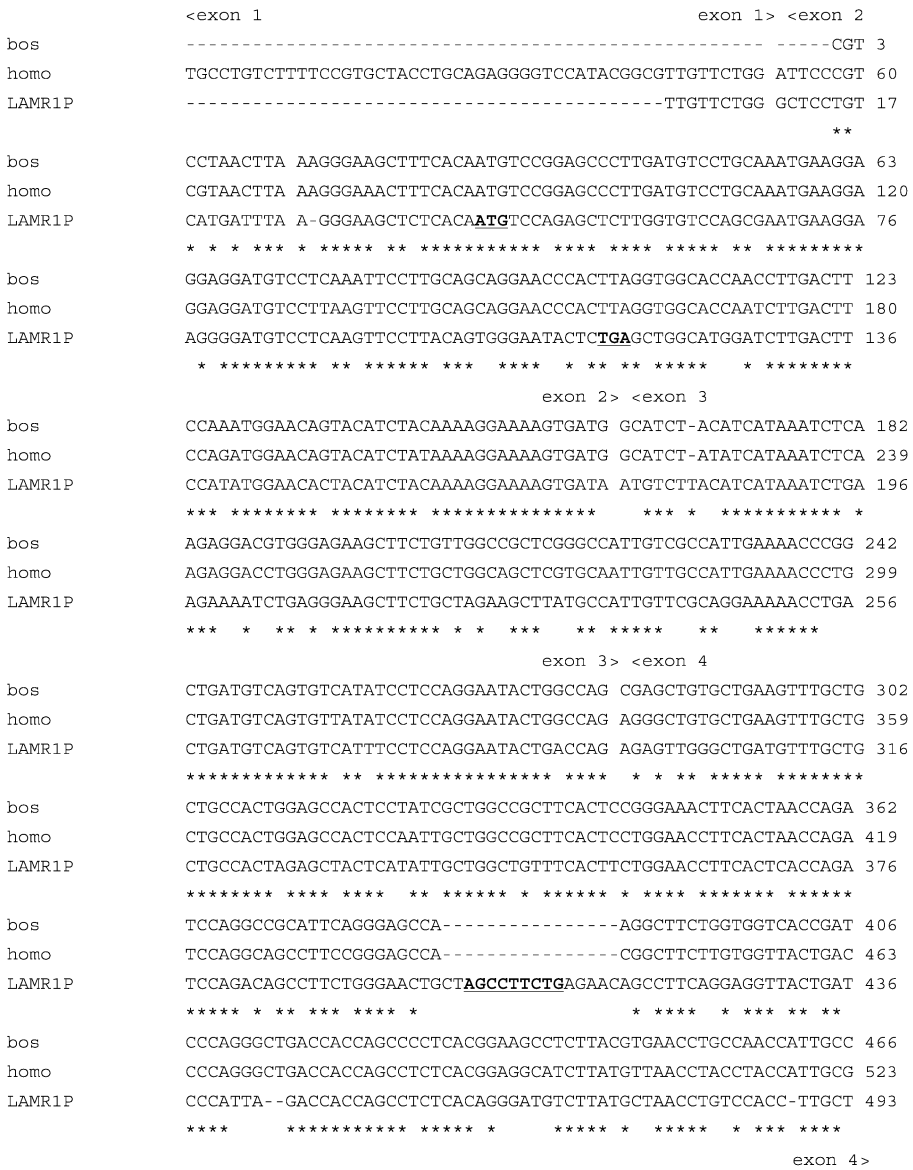


Fig. 1. Alignment of the 37 kDa LR/LRP mRNA, the human 37 kDa LR/LRP mRNA and the bovine LAMR1P sequence. The putative start codon, the premature stop codon, the duplication AGCCTTC, and the 3′ poly A tail are bold and underlined in the LAMR1P sequence.

nism involved in transmission and the contact between PrPsc and the central nervous system, the major site of TSE/BSE pathology are also still unknown. The 37 kDa LRP is suspected to play an important role in all these processes.

The genomic sequence of bovine LR/LRP is so far unknown, while the human LR/LRP gene is characterized and consists of 8,171 bp organized in 7 exons and 6 introns (Jackers et al., 1996; GenBank Acc. No. HSU43901). Grosso et al. (1991) earlier published a sequence representing the bovine 37 kDa LR/LRP mRNA (GenBank Acc. No. NM_174379). Further mammalian LR/LRP sequences are described for *Mus musculus* and *Rattus norvegicus* (Strausberg et al., 2002; GenBank Acc. Nos. BC055886 and BC060578). Comparisons of the mammalian LR/LRP sequences reveal a high level of conservation between species (Rao et al., 1989).

Here we report the characterization and chromosomal assignment of a bovine LR/LRP pseudogene.

Materials and methods

BAC library screening and sequencing of a BAC clone

Initially – with the intention to isolate the bovine functional 37-kDa LRP gene – a genomic BAC Library (Bov Bac II, No. 754; Buitkamp et al., 2000) was screened by PCR. To do so, primers (LAMEx4-1: 5′-GCTGTGCTGAAGTTTGCTGCT-3′ and LAMEx4-2: 5′-CATGGGATGGCAATGTCCACA-3′) were designed based on human genomic 37 kDa LRP sequence

```
                <exon 5
bos              GGAGCGCACTCAGTGGGTCTGATGTGGTGGATGCTCGCCCGGGAAGTCCTGCGCATGCGT 586
homo             GGAGCTCACTCAGTGGGTTTAATGTGGTGGATGCTGGCTCGGGAAGTTCTGCGCATGCGT 643
LAMR1P           GGAACTCACTCAGTGGATCTGA-GTGGTGGATGCTAGCTCAGGAGGCTCTGCACATGTGT 612
                 *** * ********** * * * ************ ** * *** *  **** **** **
bos              GGCACCATCTCC ---------------------------------------------- 598
homo             GGCACCATTTCC ---------------------------------------------- 655
LAMR1P           GGTACCATTTCC ATGGTAGTAACACGACTGAGCGACTTCACTTTGACTTTTCACTTTCAT 672
                 ** ***** ***
bos              ----------------------------------------CGAGAGCACCC-----GTG 612
homo             ----------------------------------------CGTGAACACCC-----ATG 669
LAMR1P           GCATTGGAGAAGGAAATGGCAACCCACTCCAGTATTCTTGCCTGGAGAATCCCAGGGATG 732
                                                        *  **  * **      **
bos              GGAGGTCATGCCGGACCTCTACTTCTA------CAGAGA--------------------- 645
homo             GGAGGTCATGCCTGATCTGTACTTCTA------CAGAGA--------------------- 702
LAMR1P           GGGGAGCCTGGTGGGCTGCTGTCTATGGGGTCGCAGAGAGTCAGACACGACTGAAAGTGA 792
                 ** *  * **   *     *   * *       ******
                                                          exon 5> <exon 6
bos              ---------------------------------- ---------TCCTGAGGAG ATTGAAAA 663
homo             ---------------------------------- ---------TCCTGAAGAG ATTGAAAA 720
LAMR1P           CTTAGCTGTAGCAGCAGCAACATCATAGGAGGT CACACCTCATCCTGAAGAG ATTGCAAA 852
                                                    ****** **** *** ***
bos              GGAAGAGCAGGCAGCAGCTGAGAAGGCTG--TGACCAAGGAGGAGTT--TCAGGGTGAAT 719
homo             AGAAGAGCAGGCTGCTGCTGAGAAGGCAG--TGACCAAGGAGGAATT--TCAGGGTGAAT 776
LAMR1P           GCAA-AATGAGCCATTGCTGAAAAGGCAGAGTGACCAAGTAGGAACTGTTCGGCGTTAAT 911
                   ** *    **    ***** ***** *  ******** ****  *  ** * ** ***
bos              GGACTGCTCCAGCTCCAGAGTTCACGGCTGCTCAGCCTGAGGTGGCAGACTGGTCTGAAG 779
homo             GGACTGCTCCCGCTCCTGAGTTCACTGCTACTCAGCCTGAGGTTGCAGACTGGTCTGAAG 836
LAMR1P           AGGCTGGTCTAGTACCTGAGTTAACTGCTATTCAAT-TGAGATCACAGAGGTATCTAGAG 970
                  * *** **  *  ** ***** ** ***  ***   **** *  ****    ***  **
                                                  exon 6> <exon7
bos              GCGTGCAGGTGCCTTCCGTGCCCATTCAGCAGTTCCCCACTG AAGACTGGAGTGCTCAGC 839
homo             GTGTACAGGTGCCCTCTGTGCCTATTCAGCAATTCCCTACTG AAGACTGGAGCGCTCAGC 896
LAMR1P           --GTTCAGGTGTTCTGGG-GCCGGTTCAGCAGT-CCCTATGG CAGGCGGAGGTGCTCAGC 1026
                   ** ******   *  * ***  ******* * *** *  *  ** * *  * *******
bos              CTTCCACTGAAGACTGGTCTGCAGCCCCCACCGCCCAGGCCACGGAATGGGTAGG AACCA 899
homo             CTGCCACGGAAGACTGGTCTGCAGCTCCCACTGCTCAGGCCACTGAATGGGTAGG AGCAA 956
LAMR1P           CCATCACTGAAGACTGGTCTGCAACCCATACTGCTCAGGTCACTAAATGGATAGG TACAA 1086
                 *   *** *************** * *  ** ** **** ***  ***** ****   * *
bos              CCACCGAGTGGTCGTAAGCTCTTCTTCCAGAC---AACTTGCAGAACTTCCACAAACTTC 956
homo             CCACTGACTGGTCTTAAGCTGTTCT--------------TGCATAGGCTC--------TT 994
LAMR1P           GC-CTGAGTAGCCTGAAGTGGTTCATTCAGACTTTAAAATAGAAAATATAAAATAGAAAT 1145
                  * * ** * * *  ***   ***               *  * *   *
                                                      exon 7>
bos              CACCAAAATGGAAATTTGGTTGATGGAAAATAAACT---GTTTCT 998
homo             AAGCAGCATGGAAAAATGGTTGATGGAAAATAAACATCAGTTTCT 1039
LAMR1P           AAGTGTGAGAGAAAG-TGTTTCTTTAAAAAAAAAAAGAATTC-- 1187
                  *     *  ****  ** **  *  **** ***      **
```

(Jackers et al., 1996; GenBank Acc. No. HSU43901). A probe of 230 bp length was amplified with the primers. PCR was performed in a total volume of 25 µl using 20 to 100 ng genomic DNA and 0.5 U Taq polymerase (Qiagen, Hilden, Germany). The selected region on the DNA was amplified by 34 cycles of PCR at 92 °C for 30 s, 56 °C for 30 s and 72 °C for 45 s. PCR was completed with a final elongation at 72 °C for 5 min. PCR screening of the BAC library led to isolation of clone TUM_B754F13110Q2, which was digested with several restriction enzymes. Restricted fragments were electrophoretically separated on a 0.8 % agarose gel and blotted onto a Hybond-N membrane (Amersham Biosciences, Freiburg, Germany). Blots were hybridized overnight at 42 °C with the non-radioactively labelled 230-bp probe (ECL Labelling Kit; Amersham Biosciences). Restriction fragments matching the audiogram pattern were excised from the gel and subcloned into the polylinker of the pGEM-4Z vector (Stratagene, Heidelberg, Germany) according to standard protocols (Ausubel et al., 1995). Sequencing of the clones was done with M13-universal (5′GTAAAACGACGGCCAGT3′) and M13-reverse (5′GGAAACAGCTATGACCATG3′) oligonucleotides as sequencing primers using an ABI Prism 3100 Genetic Analyser (Applied Biosystems, Weiterstadt, Germany). Sequences were trimmed to generate overlapping contigs using the software Sequencer 4.1 (Gene Codes, Ann Arbor, Mich., USA). Primer walking was used to close remaining sequence gaps and to ensure complete sequence for both strands. Sequence data were then subjected to BLAST analysis (Altschul et al., 1997) and processed using program ClustalW at EMBL (http://www.embl.de/). The nucleotide sequence reported in this paper has been deposited with the GenBank Nucleotide Sequence Database under accession number AY462060.

Fluorescence in situ hybridization (FISH)

FISH was performed as described previously by Toldo et al. (1993) and Solinas-Toldo et al. (1995) using bovine metaphase spreads (prepared from peripheral lymphocytes) obtained from a normal, healthy bull. Prior to FISH, the QFQ-banded spreads were photographed using a cooled CCD camera. Hybridization signals were detected and amplified by incubation with Streptavidin-Cy3 (Rockland, Gilbertsville, USA). The chromosomes were then DAPI-counterstained (Sigma, Deisenhofen, Germany). The relative positions of the signals on the chromosomes were measured considering the distance to the telomere and the length of the entire chromosome enabling the calculation of the fractional length (Flqter).

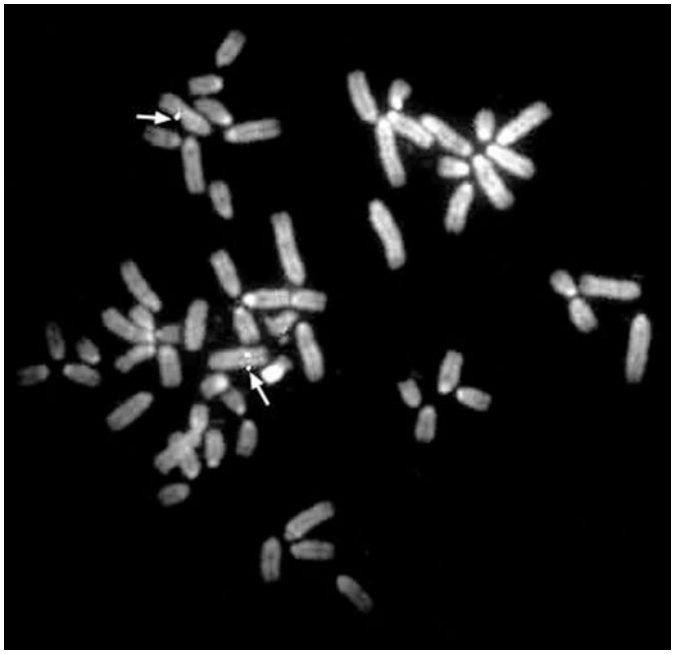
Radiation hybrid (RH) panel analyses

To determine the chromosome location of the clone, a bovine radiation hybrid panel was analysed (Williams et al., 2002; Research Genetics, Huntsville, USA). Briefly, the RH panel of 94 clones was originally created by exposing bovine fibroblast cells to 3,000 rad of X-ray and fusing the irradiated cells with non-irradiated HPRT-deficient hamster recipient cells (Wg3H). A pair of primers (RH3: 5′CTCTGCACATAGTGTGGTAC3′, and RH4: 5′TTGCAATCTCTTCAGGATGA3′), deduced from the obtained bovine sequence was used for PCR amplification of a 255-bp fragment. The panel DNAs were independently amplified twice in a reaction volume of 25 µl using 25 ng DNA and 0.5 U Q-Biogene Taq polymerase (Heidelberg, Germany) at an annealing temperature of 55 °C. PCR products were separated on a 1 % agarose gel. Two persons independently scored PCR products and finally the RHMAP3.0 package (Lange et al., 1995) was used for a two-point analysis of the marker against approximately 1,200 bovine microsatellite markers already placed on the RH map.

Results and discussion

Automated DNA sequencing of the clone was performed to achieve a consensus sequence comprised of 2,901 bp (GenBank Acc. No. AY462060). A total of 1,746 bp immediately starting with the 5′-end of the sequence revealed no homologies to any reported laminin receptor gene sequence, whereas the following 1,155 bp aligned specifically with laminin receptor sequences of several species (Fig. 1). TUM_B754F13110Q2 isolated to contain the bovine laminin receptor gene was finally confirmed to harbor a processed pseudogene sequence, which was named laminin receptor pseudogene 1 (LAMR1P). Several characteristics specific for processed pseudogenes were detected in the sequence (Vanin, 1984; Ophir and Graur, 1997): First, the multiple alignment of LAMR1P, the bovine LR/LRP mRNA and the human LR/LRP cDNA (Fig. 1) provided evidence that LAMR1P contains no intron sequences. According to that observation, we defined exon-like regions throughout the sequence at positions 1 to 9 (exon 1), at positions 10 to 174 (exon 2), at positions 175 to 294 (exon 3), at positions 295 to 553 (exon 4), at positions 554 to 844 (exon 5), at positions 845 to 1008 (exon 6), and finally at positions 1009 to 1187 (exon 7). Several transversions and transitions as well as small insertions (<3 nucleotides) but only a few deletions exist between the bovine LR/LRP mRNA and LAMR1P. Apart from that, complex insertions (>3 nucleotides) were found in the exon-4-like and exon-5-like sequence segments (e.g. positions 400 to 415, 625 to 713, 760 to 765, and 772 to 834). Motif AGCCTTC at nucleotides 402 to 408 is hereby a sequence duplication of the base stretch 416 to 422, which corresponds to the coding region in the functional gene. Translation start in both the bovine and the human laminin receptor gene is located in the respective exon 2 (Grosso et al., 1991; Jackers et al., 1996). An ATG motif was also present in the exon-2-like sequence of LAMR1P between nucleotides 42 and 44 (Fig. 1). However, at position 115 a T→G transversion creates a stop codon motif (TGA). This sequence alteration would prematurely terminate the translation of a potential open reading frame. Moreover, a 3′-poly A tail was found at the end of LAMRP1 (position 1170 to 1181), which replaces the functional polyadenylation site, necessary for the formation of an intact 3′ terminus. Using the EMBL program Repeat Masker (http://woody.embl-heidelberg.de/repeatmask/) a percentage of 46.55 of repetitive elements was identified in the exon-like sequence regions (14.9 % are SINE elements and 31.65 % are LINE elements). Comparison of the LAMR1P sequence of 1,155 bp with the bovine mRNA revealed an overall nucleotide similarity of 58.5 %. LAMR1P shows a proportion of A + T of 51.72 %.

RH mapping analysis by PCR was carried out with primers designed from parts of the bovine sequence with no homologies to any other known laminin receptor sequence in order to avoid possible cross amplifications to the human 37 kDa LR/LRP sequence. Present (1) versus absent (0) scoring of PCR results (11100 10100 00010 01001 01001 01000 11000 00000 01000 00000 00100 10001 00100 10000 00001 01000 00000 00000 0000) and the following two-point analysis revealed close linkage of LAMR1P to marker BM6458 at a distance of 19 cR (LOD score 11.6). This marker has previously been mapped to BTA4 (Bishop et al., 1994). Chromosome location of LAMR1P was further confirmed by fluorescence in situ hybridization (Fig. 2) with the most precise location at BTA4q24→q26 (number of chromosomes measured: 28; examined metaphases: 15; Flqter: 0.38 ± 0.05). Processed pseudogenes derive from mRNA presumably from retrotransposition-like integration events. Several reports indicate the lack of synteny between processed pseudogenes (Vanin, 1984) and their productive counterparts, although they are more likely to persist in loci where they do not cause deleterious effects (Mighell et al., 2000). Besides the functional gene on chromosome HSA3p21.3 – homologous bovine chromosomes are BTA1 and BTA22 – at least 26 processed human laminin receptor pseudogenes exist, which are indeed randomly distributed across the genome (Jackers et al., 1996). None of the processed pseudogenes is assigned to a human chromosome region homologous to BTA4q24→q26. If the genome structure in cattle is similar to humans, further processed laminin receptor pseudogenes are expected in cattle. First, the BAC-library screen with the described oligonucleotides leading to the isolation of LAMR1P also revealed several (weaker) signals in further clones. Secondly, the laminin receptor is a multifunctional gene, thus transcriptionally very active (Jackers et al., 1996). A large number of processed pseudogenes belonging to a family are regarded as an indicator for an increased transcription of the productive gene in a cell (Vanin, 1984). Finally, although the knowledge of vertebrate genome organization is still vague, results as presented by Dunham et al. (1999) indicate that more pseudogenes than calculated have to be expected. Exemplarily for human chromosome 22, they report that 19 % of the coding region represented pseudogenes, 82 % of these were processed pseudogenes. Thus, the isolation of the productive gene from any gene family is challenging. Mighell et al. (2000) reviewed for humans



the situation that one processed pseudogene called LAMRL5 reveals a nucleotide identity of 97.5% with the functional gene. Considering the coding sequence of the human LR/LRP gene it means that the two genes differ by only 22 bp, which hampers strategies to isolate the productive gene from gene libraries. Here the construction of the cDNA sequence and the comparison with existing pseudogene sequences is helpful to detect regions revealing the most alterations. Primers defined there – which also span intron regions – are more likely to amplify the functional gene as demonstrated by Jackers et al. (1996) for the human LR/LRP gene. Comparison of LAMR1P and the bovine LR/LRP mRNA indicate highest identities between the regions representing exon 2 and exon 3 (respectively exon-2- and exon-3-like segments) which will thus be omitted in any further primer definition.

Fig. 2. Chromosomal assignment of bovine LAMR1P by fluorescence in situ hybridization (FISH). Signals were detected on chromosome BTA4.

References

Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ: Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25:3389–3402 (1997).

Ausubel F, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl K: Short Protocols in Molecular Biology, 3rd Ed (Wiley Publishers, New York 1995).

Bishop MD, Kappes SM, Keele JW, Stone RT, Sunden SL, Hawkins GA, Toldo SS, Fries R, Grosz MD, Yoo J: A genetic linkage map for cattle. Genetics 136:619–639 (1994).

Buitkamp J, Kollers S, Durstewitz G, Fries R, Welzel K, Schäfer K, Kellermann A, Lehrach H: Construction and characterization of a gridded cattle BAC library. Anim Genet 31:347–351 (2000).

Buto S, Tagliabue E, Ardini E, Magnifico A, Ghirelli C, van den Brule F, Castronovo V, Colnaghi MI, Sobel ME, Menard S: Formation of the 67-kDa laminin receptor by acylation of the precursor. J Cell Biochem 3:244–251 (1998).

Castronovo V, Claysmith AP, Barker KT, Cioce V, Krutzsch HC, Sobel ME: Biosynthesis of the 67 kDa high affinity laminin receptor. Biochem Biophys Res Commun 177:177–183 (1991).

Dunham I, Shimizu N, Roe BA, Chissoe S, Hunt AR, et al: The DNA sequence of human chromosome 22. Nature 402:489–495 (1999).

Grosso LE, Park PW, Mecham RP: Characterization of a putative clone for the 67 kilodalton elastin/laminin receptor suggests that it encodes a cytoplasmic protein rather than a cell surface receptor. Biochemistry 30:3346–3350 (1991).

Jackers P, Minoletti F, Belotti D, Clausse N, Sozzi G, Sobel ME, Castronovo V: Isolation from a multigene family of the active human gene of the metastasis-associated multifunctional protein 37 LRP/p40 at chromosome 3p21.3. Oncogene 14:627 (1996).

Lange K, Boehnke M, Cox DR, Lunetta KL: Statistical methods for polyploid radiation hybrid mapping. Genome Res 5:136–150 (1995).

Mighell AJ, Smith NR, Robinson PA, Markham AF: Vertebrate pseudogenes. FEBS Letters 468:109–114 (2000).

Ophir R, Graur D: Patterns and rates of indel evolution in processed pseudogenes from humans and murids. Gene 205:191–202 (1997).

Protopopova EV, Konavalova SN, Loktev VB: Isolation of a cellular receptor for tick-borne encephalitis virus using anti-idiotypic antibodies. Vopr Virusol 42:264–268 (1997).

Prusiner SB: Novel proteinaceus infectious particles cause scrapie. Science 216:136–144 (1982).

Rao CN, Castronovo V, Schmitt MC, Wewer UM, Claysmith AP, Liotta LA, Sobel ME: Evidence for a precursor of the high-affinity metastasis-associated murine laminin receptor. Biochemistry 28:7476–7486 (1989).

Rieger R, Lasmezas CI, Weiss S: Role of 37 kDa laminin receptor precursor in the life cycle of prions. Transfus Clin Biol 6:7–16 (1997).

Solinas-Toldo S, Mezzelani A, Hawkins GA, Bishop MD, Olsaker I, Mackinley A, Ferretti L, Fries R: Combined Q-banding and fluorescence in situ hybridization for the identification of bovine chromosomes 1 to 7. Cytogenet Cell Genet 69:1–6 (1995).

Strausberg RL, Feingold EA, Grouse LH, Derge JG, Klausner RD, Collins FS, Wagner L, Shenmen CM, Schuler GD, et al: Generation and initial analysis of more than 15,000 full-length human and mouse cDNA sequences. Proc Natl Acad Sci USA 99:16899–16903 (2002).

Toldo SS, Fries R, Steffen P, Neibergers HL, Barendse W, Womack JF, Hetzel DJ, Stranzinger G: Physically mapped, cosmid-derived microsatellite markers as anchor loci on bovine chromosomes. Mammal Genome 4:720–727 (1993).

Vanin EF: Processed pseudogenes: Characteristics and evolution. Biochim Biophys Acta 782:231–241 (1984).

Wang KS, Kuhn RJ, Strauss EG, Ou S, Strauss JH: High-affinity laminin receptor is a receptor for Sindbis virus in mammalian cells. J Virol 66:4992–5001 (1992).

Williams JL, Eggen A, Ferretti L, Farr CJ, Gautier M, Amati G, Ball G, Caramorr T, Critcher R, Costa S, Hextall P, Hills D, Jeulin A, Kiguwa SL, Ross O, Smith AL, Saunier K, Urquhart B, Waddington D: A bovine whole-genome radiation hybrid panel and outline map. Mammal Genome 13:469–474 (2002).

Yow HK, Wong JM, Chen HS, Lee CG, Davis S, Steel GD Jr, Chen LB: Increased mRNA expression of a laminin-binding protein in human colon carcinoma: complete sequence of a full-length cDNA encoding the protein. Proc Natl Acad Sci USA 85:6394–6398 (1988).