MOLECULAR BIOLOGY

Locus-Specific Diagnostic Tests for Endogenous Avian Leukosis-Type Viral Loci in Chickens

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ABSTRACT

The genome of the chicken, Gallus gallus, contains endogenous proviral elements (ALVE elements or ev genes) that display a high degree of similarity to the Avian Leukosis class of retroviruses. The ALVE proviruses are known to modulate physiological processes of the host birds. Different ALVE elements retain variable portions of the complete, prototype viral genome, and each provirus resides in its own specific location within the host genome. Thus, each ALVE element has its own particular potential to modulate host physiology depending on the nature of its integration site, the completeness of the proviral genome, and the level of expression of the locus. It is important, therefore, to be able to establish the ALVE element profiles of chickens quickly and accurately, both in the laboratory and in a commercial setting. The current method of choice for simple, quick, and accurate typing is the polymerase chain reaction (PCR). This paper reviews the present status of PCR typing of ALVE proviruses and lists the assay protocols for 19 different elements. In addition, it compares the insertion sites of these elements in an effort to identify common motifs at ALVE integration sites.

(Key words: endogenous avian retrovirus, polymerase chain reaction diagnostic tests, proviral integration sites)

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INTRODUCTION

The genomes of most chickens harbor genetic elements belonging to the Avian Leukosis (ALV) group of retroviruses (see Crittenden, 1991, for a review). This paper employs the designation ALVE proviruses, adopted by the 1994 ISAG Workshop on Avian Endogenous Viruses, in order to differentiate the ALV-type endogenous proviruses (also known as ALV subgroup E proviruses, or ev genes) from elements belonging to other families of endogenous retroviral loci in the chicken genome.

The endogenous ALVE loci are similar in structure to the exogenous leukemia viruses, which are pathogens of chickens and related species of birds. Some endogenous proviral loci retain the capacity to code for complete, infectious retroviruses that are capable of spread by standard routes of transmission. Many apparently complete elements are inactive due to hypermethylation or position effects. Other proviral loci have undergone internal deletion and encode only a subset of the peptides that are necessary to produce an infectious viral particle. The crucial difference between endogenous retroviruses and their exogenous counterparts lies in the types of host cells that they are able to infect. Whereas exogenous viruses propagate in somatic cells only, endogenous retroviruses have, at some point in time, infected the germ line cells of the host and, once stably integrated, are inherited in a Mendelian manner along with the rest of the host genome.

Interest in ALVE proviruses stems from their effects on the physiology of the carrier chickens. A number of studies have documented the effects of ALVE elements on disease resistance, in particular the response of ALVE carriers to infection by exogenous ALV (e.g., see Gavora et al., 1995). In addition, there is considerable experimental evidence that documents changes in the frequencies of certain ALVE loci in response to selection for specific production traits (Gavora et al., 1989, 1991; Kuhnlein et al., 1989a,b; Lamont et al., 1992; Sabour et al., 1992). Although it has been shown that ALVE elements are nonessential genetic components of the chicken genome (Astrin et al., 1979), the average modern egg bird carries 1 to 3 elements, and meat birds typically carry many more proviruses (6 to 10 per bird). Thus, the ability to...
establish the ALVE locus profiles of birds quickly and accurately is important both in experimental and in commercial populations.

The total number of ALVE elements in the Gallus lineage remains to be established; however, current evidence suggests that there are over 50 different ALV-type endogenous loci. Of this total, the first 22 ALVE loci analyzed were from White Leghorn chickens, and a number of these elements have been characterized in some detail (Crittenden, 1991). Brown Leghorns, broiler lines, and exotic breeds are known to carry numerous ALVE loci, and several preliminary studies have been carried out to characterize the elements in these lines at the restriction fragment length polymorphism (RFLP) level (Gudkov et al., 1986; Aarts et al., 1991; Boulliou et al., 1991; Iraqi et al., 1991; Ronfort et al., 1991; Sabour et al., 1992; Tixier-Boichard et al., 1994a; Grunder et al., 1995). The available data show that some of the ALVE loci found in breeds other than the White Leghorn are equivalent to elements that are among the first 22 characterized in White Leghorn chickens, whereas others appear to be novel proviruses.

Prior to the development of the polymerase chain reaction (PCR) technique (Mullis and Faloona, 1987), Southern blot analysis was the method employed for the identification of env elements. Both retroviral element-specific DNA fragments and, more recently, flanking region-derived, locus-specific probes were used in RFLP analysis. Essentially, the RFLP technique discriminates between different elements based on the distribution of restriction endonuclease recognition sequences in the DNA of the chicken genome upstream and downstream of the proviral insertion site. In practice, Southern analysis is relatively expensive and time-consuming. In addition, the ability of the Southern assay to discriminate between elements is somewhat limited, particularly for bands that are larger than 10 kb. As a result, the current method of choice for element identification is PCR-based analysis using locus-specific, diagnostic tests (Benkel et al., 1992).

The PCR-based tests make use of oligonucleotide primers that target the regions flanking the viral element (the unoccupied site) and portions of the viral element itself to generate distinct amplified fragments for both the unoccupied and occupied sites (see Figure 1 for schematics of typical PCR tests). The amplified products of the multiplex, single-tube reaction can easily be separated on agarose gels. Moreover, typing of birds for carrier status, including discrimination between homozygotes and heterozygotes, is simple and unambiguous. When combined with rapid methods for the preparation of substrate (Higuchi, 1989), a typical PCR-based diagnostic procedure, starting with a blood sample and ending with a diagnostic gel, can be carried out in a single working day.

Over the past 7 yr, my laboratory has collaborated in the development of locus-specific, PCR-based assays for a total of 19 ALVE loci. The primary purpose of this report is to consolidate the currently available information on PCR tests for ALVE endogenous loci into a single source. In addition, the development of the locus-specific assays necessitated the molecular cloning and DNA sequencing of the portions of the chicken genome that harbor these elements (the unoccupied sites). A comparison of the known unoccupied sites for ALVE elements revealed no obvious similarities in nucleotide sequence; however, many ALV-type endogenous proviruses appear to be inserted into repetitive-sequence, noncoding regions of the chicken genome.
**LOCUS-SPECIFIC PCR TESTS**

**PCR Tests for Endogenous ALVE Elements**

The conditions given in Appendix 1 for the PCR tests are standardized for the Perkin Elmer Thermocycler, using a 50-μL reaction mixture in the standard 500-μL tubes, overlaid with oil. The tests are normally carried out using 50 to 350 ng of high molecular weight DNA, or the equivalent of 0.05 μL of whole blood processed using the quick substrate preparation method (Higuchi, 1989), and 50 pmol of each primer, in the standard Perkin Elmer reaction mixture. For complete PCR tests, increasing the LTR-specific primer concentration to 100 pmol per reaction usually improves the overall result fractionally.

The basic PCR profile begins with a 2 min incubation at 96°C to denature the template DNA, followed by a PCR cycle program consisting of: 1 min at 95°C, 1 min at the specified annealing temperature (Appendix 1), and 2 min at 72°C, for the number of cycles indicated; followed by a final extension at 72°C for 10 min. The amplification profiles used a modified touchdown PCR approach (Don et al., 1991) in order to minimize background due to nonspecific primer annealing. In the touchdown procedure, the annealing temperature of the PCR cycle program starts out high (near the calculated melting temperature of the PCR primers) and decreases sequentially with increasing numbers of cycles.

In addition to the primer sequences for the locus-specific tests, the profile parameters, and the sizes of amplified products, Appendix 1 also indicates whether an assay has been published previously and in what way, if any, the version given in the table differs from the previously published test. As indicated above, the profiles shown are optimized for the Perkin Elmer Thermocycler, however they can also be adapted for use on the Perkin Elmer 9600, or equivalent PCR machines, by adjusting the PCR cycle program incubation times. As a rule of thumb, for the Perkin Elmer 9600 we routinely shorten the incubation times for the denaturation and primer annealing steps to 30 s, and the extension steps within the cycle program to 90 s. All other parameters remain the same as for the older machine.

Complete PCR assays (see Figure 1A) have a built-in internal control function, i.e., a blank lane represents a failed reaction that must be repeated. Partial PCR tests (see Figure 1B) must be run in conjunction with a control reaction. Essentially any PCR test for a specific chicken gene can serve as a control reaction; we routinely use a test for the apoVLDLII gene (see Gavora et al., 1995). The control reaction can either be run in parallel in a separate tube, or by multiplexing reactions within the same tube. In our experience, it is usually easier to run the primary and control reactions for the partial tests in separate tubes, as this avoids the time-consuming process of optimizing the concentrations of the primers for the control fragment. Moreover, when screening the same bird for a number of different ALVE loci using both partial and complete PCR tests, the complete PCR assays serve as control reactions for the partial tests included within the series.

The PCR amplification products are analyzed on standard agarose gels and visualized by ethidium bromide staining (Figure 2). The separation of the PCR products for most of the locus-specific tests can conveniently be performed on 2% gels; however, there are several tests, e.g., ALVE9 and ALVE-5, for which the smaller amplified band is just over 100 bp. For these assays, increasing the agarose percentage to 2.5% and lengthening the electrophoresis time helps to separate the shorter band from any unincorporated PCR primers. Most of the complete tests are designed such that the smaller amplified band is derived from the occupied site. However, there are a number of exceptions to this rule, notably ALVE7, ALVE15, and ALVE16.

According to the available data, the loci listed in Appendix 1 have conserved the integrity of the sequences of both the insertion site and the proviral element itself over the evolutionary history of the domestication of the modern chicken. The exception is the ALVE21/slow-feathering complex, for which polymorphisms have been described for both the flanking regions and the proviral locus (Tixier-Boichard et al., 1997). In fact, in this case two distinct proviral inserts can be found at essentially the same location within the genome of the chicken. The two inserts differ at their 5' ends; i.e., whereas the slow-feathering-associated ALVE21 (also known as the occupied site, OS) is a complete proviral element, the deleted occupied site locus, OSD, which was originally described in very-late feathering birds, is deleted up to roughly the

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30 mM Tris HCl pH 9.0, 50 mM KCl, dNTPs each at 200 μM, 1.5 mM MgCl₂, 0.01% gelatin, 0.1% Triton X-100, and 1.25 units of Taq DNA polymerase.
middle of the pol gene. In addition, OSD has lost about 650 bp of upstream flanking sequence. For this reason, it is necessary to perform two separate, side-by-side reactions to type birds for their OS/OSD status. In one test, the downstream portion of the standard ALVE21 assay is used to establish the overall status of the bird for unoccupied (US) sites and OS. A second reaction that targets the upstream junction region discriminates between the OS and OSD loci (see Appendix 1).

**ANALYSIS OF INSERTION SITES**

**ALVE Element Insertion Sites**

The insertion of retroviruses into the genomes of their host cells is generally considered to be a random phenomenon. However, there is evidence that retroviruses can, under some conditions, show preferred sites of integration (Shih et al., 1988). Moreover, of the one dozen ALV-type elements that have been localized within the chicken genome, roughly half are clustered on chromosome 1 (Tereba, 1983; Crittenden, 1991). This apparently nonrandom distribution has led to speculation regarding both the mechanism (retrotransposition vs gene duplication) as well as the site specificity of the ALVE integration process.

One way to determine whether ALVE proviruses preferentially integrate at particular locations within host cell DNA is to compare the sequences of the unoccupied integration sites. Table 1 shows the nucleotide sequences surrounding the insertion sites of 18 ALVE elements. Included are the hexanucleotide motifs of host DNA that are duplicated immediately upstream and downstream of each ALVE element during the integration event (Hishinuma et al., 1981). Clearly, the target site for each provirus is unique; there is no common sequence motif immediately at the insertion sites for these elements. Thus, at the nucleotide level, the insertion of ALVE proviruses appears to be a random phenomenon.

The amount of nucleotide sequence information that is available for the regions surrounding the insertion sites of the various ALVE elements varies from a minimum of 82 bp of the upstream flank for ALVE2 to a maximum of 6,897 bp of US for ALVE3. A search of the known insertion site sequences against the GenBank database, using the blastn and blastx functions (Altschul et al., 1990), revealed the following: 1) the 79 bp at the 5' end of the upstream flanking sequence for ALVE1 (Hishinuma et al., 1981) are 64% similar to an Alu-like repeat in bovine genome (Szmraj et al., 1995); 2) ALVE3 is located within intron F of the chicken proto-onco gene hck (Benkel et al., 1995); 3) the function of the insertion site of ALVE4 has not been determined; however, sequence analysis of the upstream flanking region of the provirus revealed that only 20 bp of the 3' terminus of the upstream long terminal repeat (LTR) remain and the upstream flanking region contains a string of 85 A residues that are not found in the US (unpublished data); 4) the incomplete provirus ALVE6 is located within a repeat element near the telomere of chromosome 1—the repeat encompasses several hundred base pairs and is found at several other locations within the chicken genome, notably the chicken aldolase B (Burgess and Penhoet, 1985) and 17.5 genes (Bernot et al., 1994); 5) the ALVE15 insertion site contains several A/T-rich nucleotide stretches (Smith et al., 1984); 6) ALVE16 is located within the pol region of a CR1 element (Burch et al., 1993; unpublished data); 7) ALVE21 is located about 650 bp
downstream of a CR1 element within a large duplicated genome segment (Iraqi and Smith, 1995); 8) the ALVE-B2 insertion site contains several A/T-rich stretches; 9) the ALVE-B3 insertion site contains a TA/TG microsatellite repeat (Benkel and Fong, 1996); 10) ALVE-B5 is located 30 bp upstream from a stretch of 28 thymidine residues; 11) all birds tested using a complete ALVE-B8 locus-specific PCR test (unpublished data), including all of the progeny resulting from matings of hemizygous parents, showed a band for the US—therefore, it appears that the insertion site for ALVE-B8 is present in at least two copies within chicken genome. The remainder of the US showed no unusual features or significant similarity to the sequences currently in GenBank.

Based on the data outlined above, ALVE proviruses appear to have inserted randomly into the chicken genome, without a strong preference for any specific sequence motif at or near the site of integration. Furthermore, each integration appears to represent a separate retro-replication event, as there is no evidence for replication-mediated duplication of flanking sequences. However, of the 18 elements analyzed, 11 are located either within or near repetitive-sequence elements; i.e., ALVE 2, 4, 7, 15, B3, and B5 are located close to poly-A, poly-T, or A/T-rich stretches, ALVE 1, 16, and 21 are located in or near other retroid elements, and ALVE 3, and 6 are located within introns of chicken transcriptional units.

The occurrence of ALVE elements at or near other repeat sequences within the host genome suggests a model of random proviral insertion, followed by selection against elements that mediate detrimental effects on host physiology through insertional mutagenesis. This process results in elements that either have a beneficial effect on host survival or that are located at “safe” locations within the host genome, e.g., pre-existing repetitive-sequence, intragenomic parasitic elements of the host genome. A prime example of this phenomenon is the element ALVE16, which is inserted into the pol region of a moderately long chicken CR1 element (CR1 elements are members of the vertebrate LINE family of retroid elements; Burch et al., 1993). It should be noted, however, that sequence information on ALVE insertion sites is presently limited, and that a more thorough analysis is necessary before any rigorous conclusions can be drawn. Some of the other families of endogenous viral elements in the chicken genome, i.e., the EAV (Dunwiddie et al., 1986) and ARTCH (Gudkov et al., 1992) families, are present at a much higher copy number than the ALVE elements. It will be of interest to determine whether EAV and ARTCH elements are also found preferentially within or near repetitive sequences, and whether any of these elements share insertion sites with members of the ALVE element family.

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**REFERENCES**


APPENDIX 1. Locus-specific PCR tests for ALVE elements

ALVE1:

\[ Ev1.up = 5'-GCA CCA AAC AAT CTA GTC TGT GC-3' \]
\[ Ev1.dwn = 5'-AAG TAC TCA CTT CTC TGA AC-3' \]
\[ LTRA = 5'-CCT GAA TGA AGC AGA AGG CTT C-3' \]

Profile: 56 C, 2 cycles; 53 C, 2 cycles; 50 C, 30 cycles.

Products: \( ALVE1+ = 295 \) bp; \( ALVE1- = 505 \) bp.

Comments: Complete PCR test, see Benkel et al. (1992). The LTR-specific primer LTRA has been substituted for original primer PR-C. The PCR profile has been modified into a “touchdown” program.

Distribution: \( ALVE1 \) is common in both egg and meat lines.

ALVE2:

\[ Ev2.up = 5'-GCA CCA CTG ATG GGA TTC TTG TTC TC-3' \]
\[ LTRE = 5'-GTG TTC GCA ATC GTT AGG GAC TC-3' \]

Profile: 65 C, 2 cycles; 63 C, 2 cycles; 61 C, 2 cycles; 59 C, 28 cycles.

Products: \( ALVE2+ = 320 \) bp; \( ALVE2- = \) no band.

Comments: This is a partial test that must be used in conjunction with an internal control.

Distribution: We have detected \( ALVE2 \) only in several individuals of ADOL line 7 provided to us by E. J. Smith.

ALVE3:

\[ Ev3.up = 5'-GAA ATG CCT GCC CCA TGC CAG TG-3' \]
\[ Ev3.dwn = 5'-CTT CTC CAG CTT CAG TGA CGC-3' \]
\[ LTRA = 5'-CCT GAA TGA AGC AGA AGG CTT C-3' \]


Products: \( ALVE3+ = 190 \) bp; \( ALVE3- = 270 \) bp.

Comments: Complete PCR test, see Benkel et al. (1995).

Distribution: \( ALVE3 \) is common in both egg and meat lines.

ALVE4:

\[ Ev4.up = 5'-TAA ATT CTA AGA CAC TGA CAA TCT-3' \]
\[ Ev4.dwn = 5'-CAT AAT TCT ACG TAG GCA ACA GCT-3' \]
\[ LTRB = 5'-ACC TGA ATG AAG CTG AAG GCT TC-3' \]

Profile: 64 C, 2 cycles; 61 C, 2 cycles; 59 C, 2 cycles; 56 C, 30 cycles.

Products: \( ALVE4+ = 280 \) bp; \( ALVE4- = 565 \) bp.

Comments: Complete PCR test, see Benkel (1995).

Distribution: \( ALVE4 \) has been detected in SPAFAS line 11, several commercial egg lines, and Ottawa meat lines 20, and 90.

ALVE6:

\[ Ev6.dwn = 5'-CAC AGC CCT ATG TGG CAC TTC AAG G-3' \]
\[ LTRA = 5'-CCT GAA TGA AGC AGA AGG CTT C-3' \]

Profile: 66 C, 2 cycles; 63 C, 2 cycles; 60 C, 30 cycles.

Products: \( ALVE6+ = 300 \) bp; \( ALVE6- = \) no band.

Comments: This is a partial test that must be used in conjunction with an internal control.

Distribution: \( ALVE6 \) is a common element that has been detected in a wide variety of chicken strains (see Tixier-Boichard et al., 1994a).

ALVE7:

\[ Ev7.up = 5'-CAC ATT TAT AAT ACA AGC ATG TTC T-3' \]
\[ Ev7.down = 5'-CAT GGC TCT GCT ACA ATG CAG TA-3' \]
\[ LTRC = 5'-TGT AGT CAA ATA GAG CCA GAG G-3' \]

Profile: 63 C, 2 cycles; 60 C, 2 cycles; 57 C, 2 cycles; 54 C, 30 cycles.

Products: \( ALVE7+ = 565 \) bp; \( ALVE7- = 330 \) bp.

Comments: A partial PCR test based on the upstream flanking region for \( ALVE7 \) was published previously, see Iraqi and Smith (1994).

Distribution: \( ALVE7 \) has been detected in individuals of ADOL line 15B (unpublished data) and a randombred White Leghorn strain (Tixier-Boichard et al., 1994a).

ALVE9:

\[ Ev9.up = 5'-TAG TGC ACA TAT AAT TTC AGA AGA G-3' \]
\[ Ev9.down = 5'-CAT TCT CCA TGC ACC TGA AGT G-3' \]
\[ LTRB = 5'-ACC TGA ATG AAG CTG AAG GCT TC-3' \]

Profile: 60 C, 2 cycles; 58 C, 2 cycles; 56 C, 2 cycles; 53 C, 30 cycles.

Products: \( ALVE9+ = 115 \) bp; \( ALVE9- = 450 \) bp.

Distribution: \( ALVE9 \) is relatively common in experimental and commercial egg lines.

3In order to save space, Table 1 lists only the annealing temperatures plus the numbers of cycles for each step of the PCR cycle programs for the individual locus-specific tests. For example, the profile listed for \( ALVE2 \) is as follows: 65 C, 2 cycles; 63 C, 2 cycles; 61 C, 2 cycles; 59 C, 28 cycles. Therefore, the complete PCR test for \( ev2 \) consists of a 2 min incubation at 96 C; followed by 2 cycles of 1 min at 95 C, 1 min at 65 C, and 2 min at 72 C; followed by 2 cycles of 1 min at 95 C, 1 min at 63 C, and 2 min at 72 C; followed by 2 cycles of 1 min at 95 C, 1 min at 61 C, and 2 min at 72 C; followed by 28 cycles of 1 min at 95 C, 1 min at 59 C, and 2 min at 72 C; followed by a 10-min extension at 72 C.
APPENDIX 1. Locus-specific PCR tests for ALVE elements

ALVE12:  
\text{Ex12.up = } 5'-\text{GAA CTT CCA AAG GAC TGA TAA GAG A-3'}  
\text{Ex12.dwn = } 5'-\text{TGT AGG CAG GAA TTG GCA ACA C-3'}  
\text{LTRD} = 5'-\text{CGC CCA TAT GTC CTT GCG TC-3'}  
Profile: 61 C, 3 cycles; 59 C, 3 cycles; 57 C, 28 cycles.  
Products: \text{ALVE12} = 200 bp; \text{ALVE12} = 320 bp.  
Comments: A partial PCR test based on the upstream flanking region for ALVE12 was published previously, see Iraqi and Smith (1994).  
Distribution: ALVE12 appears to be an uncommon element.

ALVE15:  
\text{Ex15.up = } 5'-\text{CAA ATG AGG GTA ATA AGG GAG-3'}  
\text{Ex15.dwn = } 5'-\text{CAC TAC CAA ATA TAA TTC TGT AG-3'}  
Profile: 58 C, 2 cycles; 55 C, 3 cycles; 52 C, 28 cycles.  
Products: \text{ALVE15} = 460 bp; \text{ALVE15} = 180 bp.  
Comments: Complete PCR test, see Benkel and Smith (1993).  
Distribution: ALVE15 is a solitary LTR that has been detected in Ottawa meat lines 20, 30, and 90, commercial broiler lines, and Barred Rock and White Wyandotte breeds, as well as ADOL line 15B1.

ALVE16:  
\text{Ex16.up = } 5'-\text{CAA TCC AGC CTG TCT AGG TAC-3'}  
\text{Ex16.dwn = } 5'-\text{GTA GTG CAC CTA GAC ATC AGC A-3'}  
Profile: 65 C, 2 cycles; 63 C, 2 cycles; 60 C, 2 cycles.  
Products: \text{ALVE16} = 630 bp; \text{ALVE16} = 355 bp.  
Comments: This is a solitary LTR which is embedded within an long CR1 element.  
Distribution: ALVE16 is carried by the line WG, which is otherwise free of endogenous ALV-type elements (Gavora et al., 1989).

ALVE21:  
\text{Ex21.up = } 5'-\text{GTG GGA ATG GTA CTA CAG AGA AGG-3'}  
\text{Ex21.dwn = } 5'-\text{CAT TTC AAG CAA GGC ACT GCC-3'}  
\text{LTRB} = 5'-\text{ACC TGA ATG GTA CTA CAG AGA GCT TC-3'}  
Profile: 66 C, 2 cycles; 63 C, 2 cycles; 60 C, 2 cycles; 57 C, 28 cycles.  
Products: \text{ALVE21} = 390 bp; \text{ALVE21} = 510 bp.  
Comments: Complete PCR test, see Tixier-Boichard et al. (1994b).  
Distribution: ALVE21 is present in sex-linked slow-feathering lines.

ALVE21*OSD  
\text{a) OSD.up = } 5'-\text{GAG TGT TCT TCA TGC AAA TAG-3'}  
\text{Ex21.dwn = } 5'-\text{CAT TTC AAG CAA GGC ACT GCC-3'}  
\text{LTR} = 5'-\text{ACC TGA ATG GTA CTA CAG AGA GCT TC-3'}  
Profile: 61 C, 2 cycles; 58 C, 2 cycles; 55 C, 2 cycles; 52 C, 28 cycles.  
Products: \text{ALVE21} = 390 bp; \text{ALVE21} = 1,060 bp.  
Comments: Complete PCR test, see Tixier-Boichard et al. (1997).  
Distribution: ALVE21*OSD is restricted to the Nunukan strain.

ALVE-B1:  
\text{Ex-B1.up = } 5'-\text{TTC GGA ACT TGA ACT TCA GCA TTC TGT T-3'}  
\text{Ex-B1.dwn = } 5'-\text{CAT TCC CTA ACG ATT GCG AAC AC-3'}  
Profile: 63 C, for 2 cycles; 60 C, for 2 cycles; 57 C, for 2 cycles; 53 C, for 30 cycles.  
Products: \text{ALVE-B1} = 200 bp; \text{ALVE-B1} = 335 bp.  
Comments: Complete PCR test, see Benkel and Grunder (1997).  
Distribution: ALVE-B1 is present in Ottawa meat lines 20 and 30.

ALVE-B2:  
\text{Ex-B2.up = } 5'-\text{GAT ATC TGT TTA CAT ACG TTA AGC CAA-3'}  
\text{Ex-B2.dwn = } 5'-\text{TTC CCA CTT GTG GTA GGC AA-3'}  
\text{LTRD} = 5'-\text{GAG TCC CTA ACG ATT GCG AAC AC-3'}  
Profile: 65 C, for 2 cycles; 62 C, for 2 cycles; 59 C, for 2 cycles; 56 C, for 30 cycles.  
Products: \text{ALVE-B2} = 165 bp; \text{ALVE-B2} = 270 bp.  
Comments: Complete PCR test, see Benkel et al. (Animal Genetics, in press).  
Distribution: ALVE-B2 is present in Ottawa meat line 90.

ALVE-B3:  
\text{Ex-B3.up = } 5'-\text{CAG TTT TCC CTA AAG GAC TAC AGA A-3'}  
\text{Ex-B3.dwn = } 5'-\text{TGC TGG AAT TCC ACA GAC TAC AGA T-3'}  
\text{LTRF} = 5'-\text{GAG TCC CTA ACG ATT GCG AAC AC-3'}  
Profile: 65 C, for 2 cycles; 62 C, for 2 cycles; 59 C, for 2 cycles; 56 C, for 30 cycles.  
Products: \text{ALVE-B3} = 293 bp; \text{ALVE-B3} = 458 bp.  
Comments: Complete PCR test, see Benkel et al. (1997).  
Distribution: ALVE-B3 is present in Ottawa meat line 90.
APPENDIX 1. Locus-specific PCR tests for ALVE elements

**ALVE-B4:**

*Ev-B4.dwn* = 5′-TTG GTT ACA TGC CAA GCA GT-3′

*LTRF* = 5′-GAG TCC CTA ACG ATT GCG AAC AC-3′

Profile: 63 C, for 2 cycles; 61 C, for 2 cycles; 58 C, for 30 cycles.

Products: *ALVE*-B4+ = 175 bp; *ALVE*-B4- = no band.

Comments: This is a partial test that must be used in conjunction with an internal control.

Distribution: *ALVE*-B4 is present in Ottawa meat lines 20 and 90.

**ALVE-B5:**

*Ev-B5.up* = 5′-CAG TCA TAT ATC CGA ATG TTT AAG TCT-3′

*Ev-B5.dwn* = 5′-GGA GCC ATA ATT TCA TAA TGA A-3′

*LTRD* = 5′-CGC CCA TAT GTC CTT GCG TC-3′

Profile: 54 C, for 2 cycles; 52 C, for 2 cycles; 50 C, for 28 cycles.

Products: *ALVE*-B5+ = 123 bp; *ALVE*-B5- = 241 bp.

Comments: Complete PCR test, see Benkel and Grunder (1997).

Distribution: *ALVE*-B5 has been found in the Albino meat line, New Hampshire, Rhode Island Red, White Jersey Giant, and White Wyandotte breeds as well as Ottawa meat lines 20, 30, and 90.

**ALVE-B6:**

*Ev-B6.up* = 5′-CCA GTC CAC ATT TCG ACT GGT T-3′

*Ev-B6.dwn* = 5′-CAG CTC AGC ACG TTG AGC TGT T-3′

*LTRF* = 5′-GAG TCC CTA ACG ATT GCG AAC AC-3′

Profile: 61 C, 2 cycles; 59 C, 2 cycles; 57 C, 30 cycles.

Products: *ALVE*-B6+ = 200 bp; *ALVE*-B6- = 340 bp.

Comments: Complete PCR test, see Benkel et al. (1996).

Distribution: *ALVE*-B6 is present in the Albino meat line, and Ottawa meat lines 20 and 30.

**ALVE-B8:**

*Ev-B8.dwn* = 5′-GAT CGA GCT CGG GGA TAA GTT TC-3′

*LTRF* = 5′-GAG TCC CTA ACG ATT GCG AAC AC-3′

Profile: 66 C, 2 cycles; 64 C, 2 cycles; 62 C, 2 cycles; 59 C, 28 cycles.

Products: *ALVE*-B8+ = 159 bp; *ALVE*-B8- = no band.

Comments: This is a partial test that must be used in conjunction with an internal control; this locus has also been described as *evb24* (Boulliou et al., 1991), *evA* (Iraqi et al., 1991), and *evR11* (Gorbovitskaia et al., in press); a PCR test for this locus has recently been published by Nave et al. (1997).

Distribution: *ALVE*-B8 has been found in Ottawa meat lines 20 and 90.