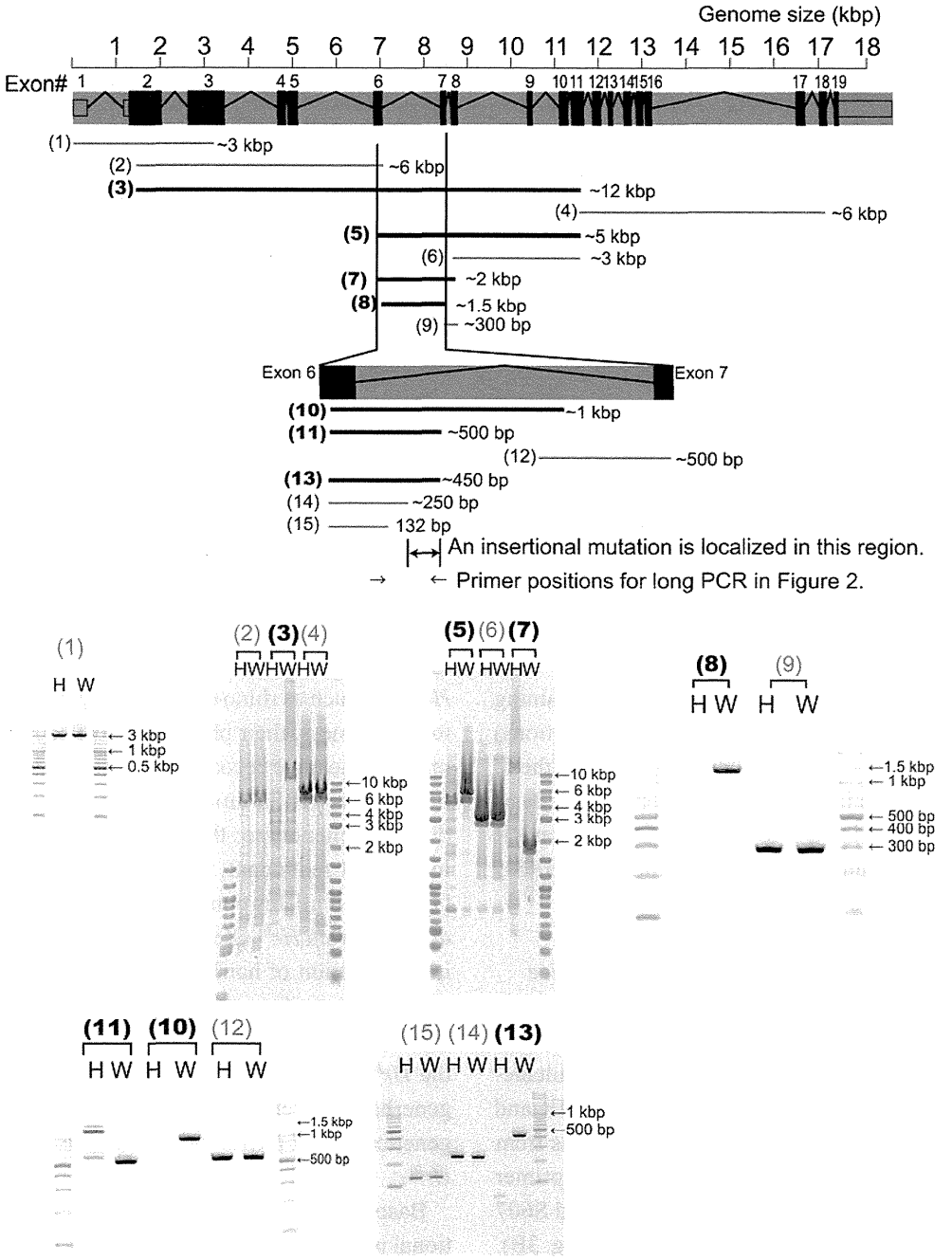

Fig. 1. Analysis of *Hr* alleles in homozygous (H) and wild-type (W) HR mice. The normal (wild-type)*Hr* allele is ~19 kbp in length and consists of 19 exons. A total of 15 overlapping PCRs covering the entire *Hr* coding sequence revealed that an insertion mutation was localized in intron 6 of the Hr^x allele. KOD FX neo was used for PCR amplification of regions 2–7, and HotStarTaq was used for PCR of other regions. PCRs shown in gray typeface (1, 2, 4, 6, 9, 12, 14, 15), no difference between homozygous and wild-type HR mice. PCRs shown in black typeface (3, 5, 7, 8, 10, 11, 13), no band or different bands were obtained in homozygous HR mice. Six agarose gel electropherograms show the band patterns of all PCRs. The primer sets used were: (1) F224 and R1151, (2) F193 and R1873, (3) F193 and R2433, (4) F2463 and R3455, (5) F1843 and R2433, (6) F2052 and R2433, (7) F1843 and R2078, (8) F1843 and R1972, (9) F1913 and R2078, (10) F1843 and Int6-R1458, (11) F1843 and Int6-R979, (12) Int6-F1290 and R1972, (13) F1843 and Int6-R850, (14) F1843 and Int6-R642, and (15) F1843 and Int6-R539. The primer sequences and elongation time are shown in Tables 1 and 2, respectively. The primer positions for long PCR shown in Fig. 2 are also indicated in this figure.

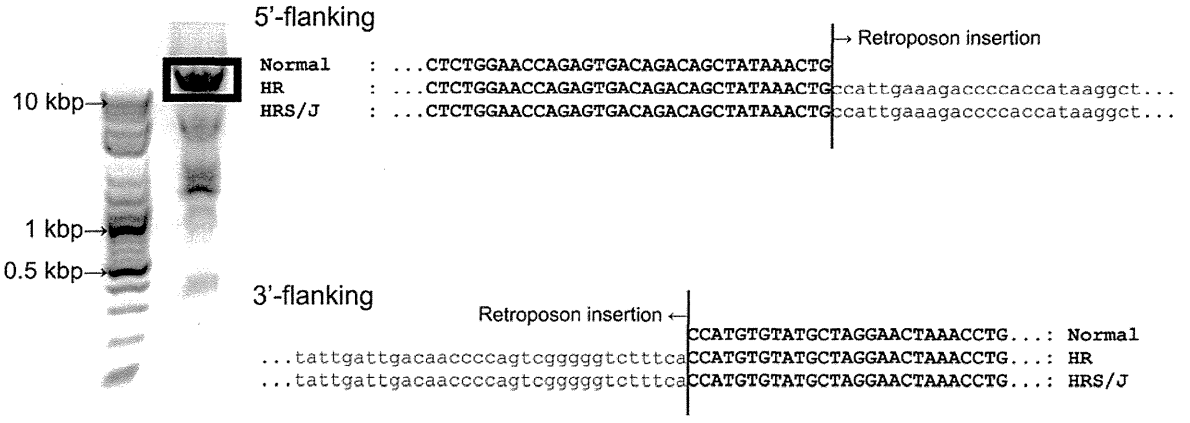

Fig. 2. Determination of DNA sequences flanking the insertion mutation in HR mice. PCR with two primers, mHR-int6-F514 and mHR-int6-R806 (positions and sequences are shown in Fig. 1 and Table 1, respectively) produced an ~13-kb-long amplicon containing insertion mutations in homozygous HR mice. Sequencing and BLAST searches indicated that the HR mice share the same insertion mutation as HRS/J mice. The sequences of the 5' and 3' regions flanking the insertion mutation of HRS/J mice were retrieved from GenBank (accession numbers M20235 and M20236, respectively).

Sequencing the mutated region in HR mice

Long PCR for amplifying genomic regions containing the insertional mutation (Fig. 1 for the primer positions) produced an ~13-kb-long amplicon (Fig. 2). Both the 5' and 3' ends of the amplicon were sequenced. Analysis of both sequences using BLAST search revealed that HR mice carried the same insertional mutation as HRS/J mice; i.e., Hr^{x} turned out to be Hr^{hr}.

Genotyping of Hr alleles in HR and Hos:HR-1 mice

Primers for genotyping *Hr* alleles were designed according to their sequence information (see Fig. 3A for primer positions). All three primers were used simultaneously for genotyping PCR. The zygosities of HR and Hos:HR-1 mice were determined using amplicons from both mutant and wild alleles with the following primer sets: S776 and R850 (275 bp, longer bands), and S607 and R850 (244 bp, shorter bands), respectively (Fig. 3B).

Discussion

Our genomic analysis revealed that the HR mice at our institute share the same hairless mutation (Hr^{hr}) as HRS/J and Skh:HR-1 (an ancestor of Hos:HR-1) mice. This indicates that the HR strain is a descendent of the original hairless mice found in London in 1924 [4]. This possibility was also suggested by the fact that the phenotype of HR mice is identical to that of other hairless mice carrying Hr^{hr} alleles. Our genomic analyses confirmed this possibility. Although other mutations of the *Hr* gene, such as rhino (Hr^{rh}) [8] and bald (Hr^{ba}) [6] lead to hairlessness, their phenotypes differ from that of HR mice. Rhino mice become completely hairless by 35 days of age, like HRS/J mice, but older rhino mice have a different phenotype: their skin becomes progressively looser and redundant, forming folds, flaps, and ridges [12]. Rhino alleles contain various types of mutations, different from Hr^{hr} alleles [1, 2]. These mutations result in a truncation of hairless proteins. On the other hand, bald mice are phenotypically intermediate between the hairless and rhino strains [6]. The similarities between the Hr^{hr} and Hr^{ba} alleles are unclear because the bald gene has not yet been sequenced. Thus, HR mice are genetically and phenotypically hairless mice that carry Hr^{hr}.

Based on the genomic sequence around the insertional mutation, we developed a PCR genotyping method. The method was confirmed to be useful for zygosity checks of both HR and Hos:HR-1 strains, and possibly more strains carrying Hr^{hr} alleles. Our three primers flanking the insertional mutation in the Hr^{hr} gene distinguished the zygosities of hairless strains in a single PCR assay. Flanking primer methods [7], often used for the genotyping of transgenes [9], are simple and precise for zygosity determination. Other methods, such as Southern blots and quantitative real-time PCR, are also used for zygosity checks, but are challenging in practice. Both methods use quantitative tests, the results of which are

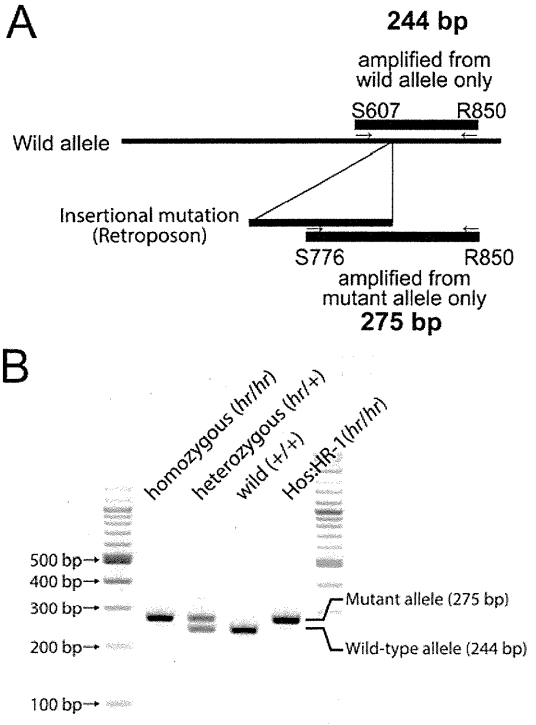

Fig. 3. PCR for genotyping *Hr* alleles in HR and Hos:HR-1 mice. (A) Primer positions (primer sequences are shown in Table 1). PCR using the primers mHR-int6-S607 (S607) and mHR-int6-R850 (R850) produces 244-bp amplicons from wild-type alleles (*Hr*, + in Fig. 3B) only. PCR using the primers mHR-int6-S776 (S776) and mHR-int6-R850 (R850) produces 275-bp amplicons from mutant alleles (Hr^{hr}, *hr* in Fig. 3B) only. (B) Zygosity determination by PCR. Zygosities of *Hr* alleles were determined by PCR using three primers (S607, S776, and R850) simultaneously. If only 275-bp amplicons were produced, the mice were taken to be homozygous (Hr^{hr}/Hr^{hr}). If only 244-bp amplicons were produced, the mice were wild-type (*Hr*/*Hr*). If both amplicons were produced, the mice were heterozygous (Hr/Hr^{hr}). Electropherograms of PCR products indicate the zygosities of homozygous, heterozygous, and wild-type HR mice as well as homozygous Hos:HR-1 mice.

often difficult to compare precisely. In contrast, the flanking primer method is based on qualitative tests (presence or absence of target amplicons) and is easy to perform with no need for complicated procedures such as hybridization of radioactive probes, precise adjustment of template concentration, and so forth. Our primers are different from those of Schaffer *et al.* [10]. Their primers targeted similar positions but had a lower Tm than our primers. We believe that our primers have an advantage because a higher Tm often leads to better results. In addition, PCR results are highly dependent on thermal controls, such as the block-temperature (i.e., based on the temperature of blocks, not that within PCR tubes) and active-tube controls (i.e., based on the temperature within PCR tubes). PCR using a thermal cycler with block temperature control needs a longer reaction time than PCR with active-tube control. In the present study, we used 10 s for the denature and annealing times in a thermal cycler with active-tube control. If a thermal cycler using block-temperature control is used, a longer period, e.g., 30 s, should be used.

Immature hairless mice can be precisely genotyped using our PCR method before their phenotype (hair coat loss) appears. This would enable the use of such mice for research that requires knowledge of precise zygosities.

In summary, the HR strain at our institute carries the same Hr^{hr} alleles as HRS/J and Skh:HR-1 (Hos:HR-1). Our genotyping method could be used for zygosity checks of various hairless mouse strains that carry Hr^{hr} alleles. This method will facilitate the study of hairless mice, and especially immature mice, the zygosities of which cannot be determined based on appearance alone.

Acknowledgment

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