Single-Strand Conformation Polymorphism (SSCP) Assays for Major Histocompatibility Complex *B* Genotyping in Chickens

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ABSTRACT We have developed a DNA-based method for defining MHC *B* system genotypes in chickens. Genotyping by this method requires neither prior determination of allele-specific differences in nucleotide sequence nor the preparation of haplotype-specific alloantisera. Allelic differences at chicken *B-F* (class I) and *B-L* (class II) loci are detected in PCR single-strand conformation polymorphism (SSCP) assays. PCR primer pairs were designed to hybridize specifically with conserved sequences surrounding hypervariable regions within the two class I and two class II loci of the *B*-complex and used to generate DNA fragments that are heat- and formamide-denatured and then analyzed on nondenaturing polyacrylamide gels. PCR primer pairs were tested for the capacity to produce SSCP patterns allowing the seven *B* haplotypes in the MHC *B* congenic lines, and seven *B* haplotypes known to be segregating in two commercial broiler breeder lines to be distinguished. Primer pairs were further evaluated for their capacity to reveal the segregation of *B* haplotypes in a fully pedigreed family and in a closed population. Concordance was found between SSCP patterns and previously assigned MHC types. *B-F* and *B-L* SSCP patterns segregated in linkage as expected for these closely linked loci. We conclude that this method is valuable for defining MHC *B* haplotypes and for detecting potential recombinant haplotypes especially when used in combination with *B-G* (class IV) typing by restriction fragment pattern.

(*Key words*: major histocompatibility complex, *B* haplotyping, single-strand conformation polymorphism pattern, major histocompatability complex class I (B-F) and class II (B-L) loci, *B-G* restriction fragment pattern)

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INTRODUCTION

MHC *B* genotype has a significant influence in how chickens respond to challenge by pathogens, particularly Marek's disease virus (Briles et al., 1977, 1983; Longenecker et al., 1977), and to vaccination as well (Bacon et al., 1981, 1983; Bacon and Witter, 1992, 1993, 1994, 1995). Many experimental and several commercial lines are routinely genotyped for MHC *B* to assure line purity and to select for particular haplotypes. All typing methods require investment of time and resources for reliable *B* haplotype determinations. For many years *B* genotypes have been determined through hemagglutination assays using specifically prepared alloantisera (Briles et al., 1982b). The hemagglutination assays are relatively simple to perform when reagents are available. But, developing and characterizing the antisera require knowledge of the genetics of the immunized animals and the availability of fully pedigreed families from the donor stock to define the specificity of each antiserum. Generally, B alloantisera are developed within individual genetic lines that are routinely typed for the several other known alloantigen systems (Briles, 1962) allowing for the selective immunization against a single *B* system antigen. Without genetic control of the other alloantigen systems, antisera against B antigens must frequently be selectively absorbed to obtain desired *B* system specificity. In addition, B-directed alloantisera typically contain antibodies to a number of epitopes associated with B-G (class IV) and B-F (class I) molecules, both of which are simultaneously expressed on individual chicken red blood cells (except for rare recombinants). Alloantisera directed against epitopes expressed on cells transfected with *B-F* class I cDNA clones provide a means of obtaining more narrowly directed reagents (Fulton et al., 2001), but these antisera also require time and effort to prepare.

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Abbreviation Key: SSCP = single-strand conformation polymorphism, rfp = restriction fragment pattern.

Alternatively, DNA-based methods can be used for MHC *B* genotyping. *B* haplotype determinations can be made through Southern hybridization with *B-G* probes. Because the *B-G* gene family is large and highly polymorphic, genetic differences in the *B-G* region are easy to detect by Southern hybridization. Restriction fragments produced with any of several restriction endonucleases provide patterns correlated with individual *B* haplotypes (Miller et al., 1988a, Uni et al., 1992, 1993; Landesman et al., 1993; Li et al., 1997; Yonash et al., 2000). One advantage of typing by *B-G* restriction fragment pattern (rfp) is that typing can begin immediately without preparation of alloantisera or DNA sequence determinations. One disadvantage of typing by *B-G* rfp is that this method currently depends on the use of ^{32}P-labeled probes and hence is limited to appropriately equipped laboratories. This method might be more widely applied if nonradioactive detection systems for DNA hybridization become sufficiently sensitive. *B-G* genes (maybe as many as 20 loci) are tightly linked with the two *B* class I (*B-FI* and *B-FIV*) and two class II (*B-L*β*I* and *B-L*β*II*) loci so frequently the *B-G* rfp can be used to define the entire *B* haplotype. Importantly, recombinant haplotypes exist, recurring occasionally, such that *B-G* alleles are sometimes found in linkage with different alleles at the *B-F* and *B-L* loci. Thus, *B-G* typing alone as performed by rfp detection or in routine hemagglutination assays is not always sufficient for identifying alleles at *B-F* and *B-L* loci. Typing *B-F* and *B-L* directly by rfp has limited application since some alleles at these loci share identical rfp. When allele sequences are available, allele specific primers can be developed so that allele-specific PCR reactions (Zheng et al., 1999) can be used as a direct means for typing *B-F* and *B-L* alleles.

The method described here for determining *B-F* and *B-L* alleles is an alternative to other techniques that has its particular advantages. SSCP (single-stranded conformational polymorphism) assays can be performed in laboratories having access to a PCR thermal cycler, equipment for PAGE and computers supporting commonly used graphics software. The SSCP patterns are detected by silver staining, eliminating the need to handle ^{32}P-labeled nucleic acids (Oto et al., 1993). The PCR primer pairs for SSCP described here were developed using well-defined *B* haplotypes and evaluated with a variety of samples representing inbred lines in which *B* alleles are fixed, and also various genetic stocks in which *B* types segregate. They can be applied immediately to produce SSCP patterns to provide information on the MHC genotypes without the need of developing additional reagents.

MATERIALS AND METHODS

Genetic Stocks Providing DNA Samples

PCR primer sets for SSCP *B-F* and *B-L* typing were tested using a variety of DNA sample sets. Four sample sets are described here. 1) DNA was obtained from individuals representing the seven MHC congenic inbred lines on the $15I_5$ background (Lamont et al., 1990) developed at ADOL (USDA ARS Avian Disease and Oncology Laboratory, East Lansing, MI) by L. D. Bacon. 2) Additional samples were obtained from homozygous individuals representing seven *B* haplotypes defined in commercial broiler breeder lines (Li et al., 1997, 1999; Zheng et al., 1999; Livant et al., 2001). Three individuals represented each haplotype. 3) DNA samples from a previously characterized fully pedigreed family (C084) in which two *B* and three *Rfp*-Y alleles are known to segregate (Briles et al., 1993) were also included. 4) Twenty additional samples were randomly selected from Charles Rivers/SPAFAS specific pathogen free line 22 White Leghorn chickens of unknown haplotype. DNA was extracted from fresh or frozen blood cells and purified as described previously (Briles et al., 1993; Li et al., 1997).

PCR Amplification

PCR reactions were performed in a Stratagene Hot-Top Robocycler[3] using HotStar Taq polymerase[4] and an initial 14-min preheating step for activation of this polymerase. Activation was followed by 30 or 35 cycles of 45 s at 94 C, 45 s at 60 C (5 C below theoretical annealing temperature (Wu et al., 1991), and 45 s at 72 C. Samples were subjected to a final 10-min interval at 72 C. Aliquots of the PCR reactions were checked for robustness before proceeding to SSCP analysis.

SSCP Analysis

One to three microliters of PCR reaction product were denatured at 80 C in 5 to 10 μL formamide/dye (1 mL formamide, 5 μL each of saturated xylene cyanol and bromophenol blue solutions) for 5 min and immediately chilled on ice to prevent reannealing. The denatured PCR products were electrophoresed for 2 to 2.5 h at 200 V (equivalent to 400 to 500 volt-h) in 10% polyacrylamide, 0.5% TBE (44.5 m*M* Tris-borate, 44.5 m*M* boric acid, 1 m*M* EDTA) gels in a Miniprotean II apparatus[5] following previously described methods (Oto et al., 1993). After trial runs at several temperatures, runs were routinely made at ambient temperature (23 to 24 C). SSCP gels for the *B-L* typing were most often run overnight at 70 V for 1200 volt-h to obtain better separation of the somewhat longer *B-L* PCR fragments. For example, the *OL284/RV280BL* patterns in Figure 2 are from a 2.5 h run and those in Figures 3, 4, and 5 are from overnight runs. The gels were fixed, stained with a Bio-Rad Silver Stain Plus Kit, packaged in gel wrap,[6] mounted in drying frames, and dried. The dried gels were scanned and images composed with Adobe Photoshop[7] and Corel Draw.[8]

[3]Stratagene, La Jolla, CA.
[4]Qiagen, Valencia, CA.
[5]Bio-Rad, Hercules, CA.
[6]BioDesign, Camel, NY.
[7]Adobe Systems Inc., San Jose, CA.
[8]Corel Corporation, Ottawa, Ontario, Canada.
[9]NEN Life Science Products, Inc., Boston, MA.
[10]Amersham Pharmacia Biotech, Piscataway, NJ.

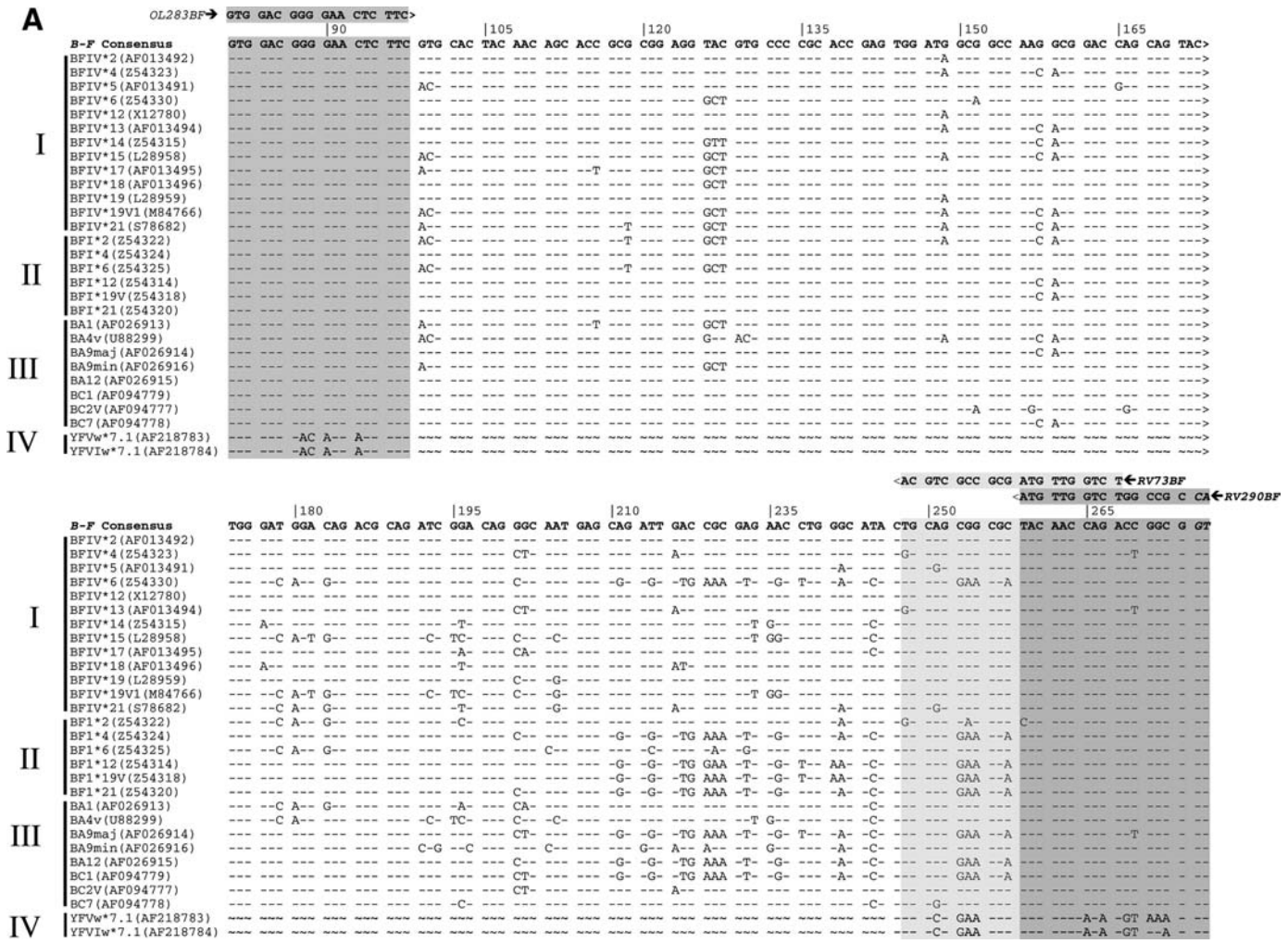

FIGURE 1. Sequence alignments for chicken MHC *B-F* (A) and *B-Lβ* (B) genes from three different sources (Groups I, II, and III) showing the positions of the SSCP priming sites above the consensus sequences. Sequences for the corresponding regions within the *Rfp-Y* genes (IV) (Miller et al., 1994, 1996; Zoorob et al., 1993) illustrate the divergence of *B* and *Rfp-Y* genes at the priming sites. The sources of the *B* system gene sequences are: White Leghorn *BFIV* and *MAJ* genes (I), White Leghorn *BFI* and *MIN* genes (II), and broiler major and minor genes with A and C notations indicating lines of origin (Li et al., 1999; Livant et al., 2001) (III). Genbank accession numbers follow allele names. Sequences are numbered starting with the first nucleotide of exon 2 as 1. Dash "–" indicates sequence identity, tilda "~" sequence not given, and dot "." sequence unknown.

B SSCP typing is technically straightforward; however consistency and attention to detail are required to routinely obtain high-quality SSCP patterns. It is important that the PCR amplification step yields a single product of the expected size. PCR amplifications within five degrees of the calculated annealing temperature (Wu et al., 1991) generally minimize mispriming on irrelevant sequences. Standardized stock reagents were used to provide consistent polyacrylamide gels. Gel rigs and the containers used for silver staining were meticulously cleaned with detergent, thoroughly rinsed, and then handled only with gloved hands. Gel plates were nitric acid cleaned. Electrophoresis at room temperature for 2 to 3.5 h was adequate for many conformers, but the longer conformers, such as those from the *B-L*-specific primers, separated better in longer electrophoresis runs at lower voltage settings.

Southern Blot Analysis

Samples containing 10 μg of genomic DNA were digested with *Pvu* II, electrophoresed in 0.8% agarose gels, transferred to Gene Screen,[9] and analyzed by Southern hybridization (Briles et al., 1993). Samples were probed with *bg11* (GenBank AF493427), a 1940 bp full length *B-G*[21] erythroid cell cDNA probe that includes the sequence of a 334 unprocessed intron located between exons 4 and 5 (Miller et al., 1988a, 1991). Images were collected using a Molecular Dynamics PhosphorImager.[10]

RESULTS

Design and Testing of B-F SSCP Primer Pairs

The two chicken *B-F* loci are especially polymorphic within exon 2, a region encoding a portion of the class I antigen binding region. Individual *B-F* alleles are easily distinguished from one another by nucleotide differences in this region as is evident in the alignment of *B-F* gene sequences in Figure 1A. Because of the variability among alleles inherent in this region, we focused on this portion

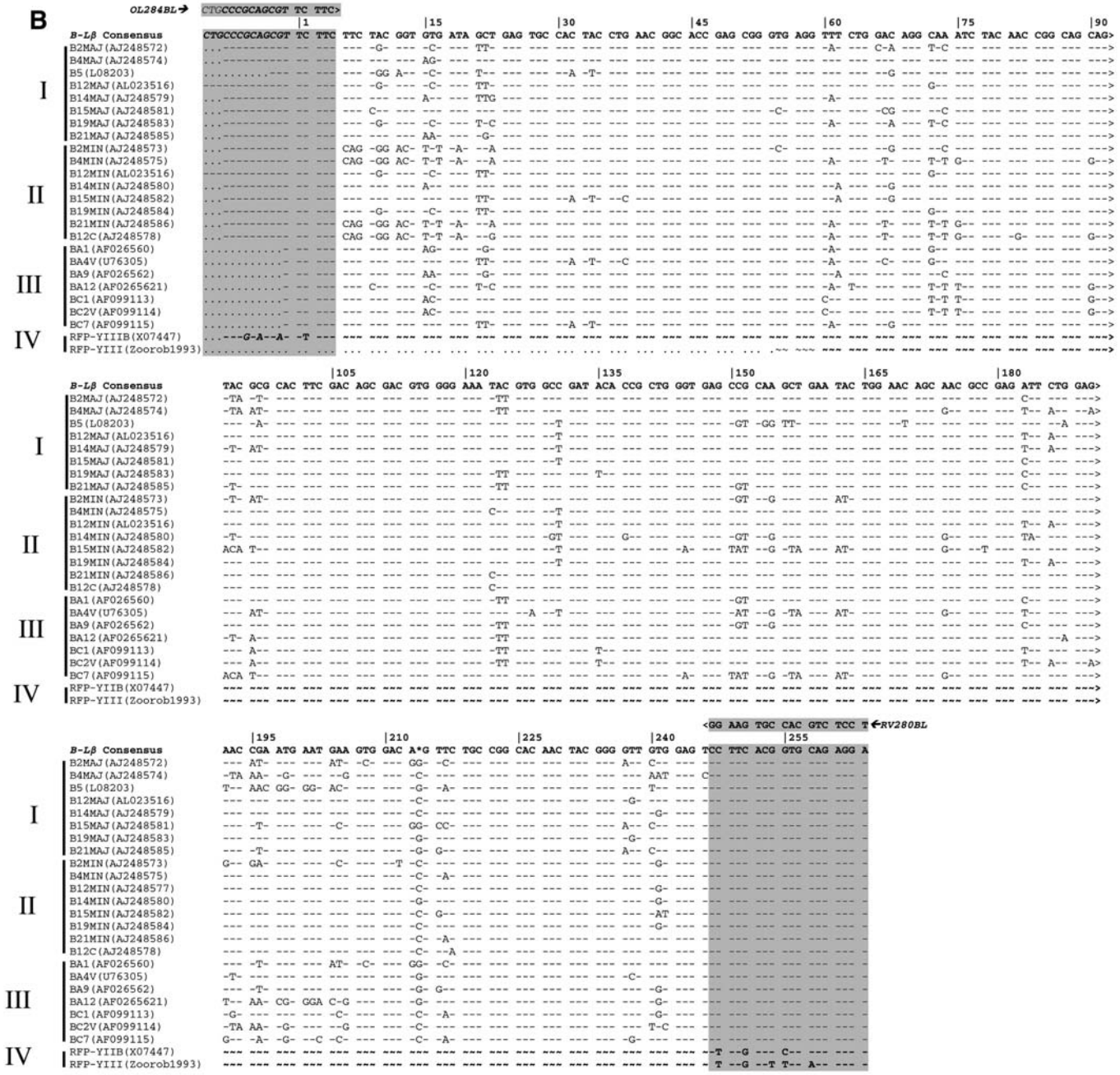

FIGURE 1. Continued.

of the *B-F* gene sequences in developing the SSCP assay. We designed and tested six primers surrounding this region in various pair combinations. Sequence sites for primers had to meet four criteria. 1) They needed to be directed toward sites conserved among known *B-F* allele sequences. As we developed this method it was necessary to design additional primers at somewhat different positions to accommodate additional *B-F* alleles as new sequences were reported. 2) The priming sites had to differ sufficiently from the equivalent sites in *Rfp-Y* class I loci (Miller et al., 1994; Afanassieff et al., 2001) to ensure PCR amplification occurs only from the *B* loci (see sequence Groups I, II, and III vs. IV in Figure 1A). 3) The amplified region within the *B* loci needed to be polymorphic in at least one of the two *BF* loci represented in a haplotype. 4) The sites had to be separated by 100 to 300 bp since PCR fragments within this range are optimal for separation in SSCP gels.

Early primer pairs, which encompassed the entire *B-F* exon 2 region (~270 bp), were found to be useful, but provided fragments somewhat difficult to routinely resolve into distinctive patterns using the short intervals of electrophoresis in our experimental conditions (data not shown). To shorten the SSCP conformer length, a forward primer was designed at a position near the middle of exon 2 that still allowed the hypervariable region within

TABLE 1. SSCP[1] primer pairs for *B-F* and *B-Lβ* genotyping

Region	Primer names	Primer length	Sequence	Anneal temperature[2]	Fragment size
B-F exon 2	*OL283BF*	18	GTG GAC GGG GAA CTC TTC	60 C	178 bp
	RV73BF	21	TCT GGT TGT AGC GCC GCT GCA		
	OL283BF	18	GTG GAC GGG GAA CTC TTC	60 C	186 bp
	RV290BF	18	ACC GCC GGT CTG GTT GTA		
B-Lβ exon 2	*OL284BL*	18	GTG CCC GCA GCG TTC TTC	60 C	277 bp
	RV280BL	18	TCC TCT GCA CCG TGA AGG		

[1]Single-strand conformation polymorphism.

[2]This temperature is approximately 5 C below the calculated theoretical hybridization temperature (Wu et al., 1991).

exon 2 to be amplified. Table 1 lists three primers found to be particularly useful in distinguishing *B-F* haplotypes (fragments originating from both *B-FI* and *B-FIV* alleles). These include a single forward primer, *OL283BF*, and two reverse primers, *RV73BF* and *RV290BF*. The use of *OL283BF* in combination with *RV73BF* was sufficient for distinguishing among many *B-F* haplotypes. The seven *B* haplotypes in the MHC *B* congenic Leghorn lines could be distinguished with this primer pair (Figure 2A). Three individuals representing each congenic line provided identical patterns. In Figure 2 the patterns for three B^{15} samples are included to illustrate reproducibility of allele specific patterns.

As more sequence data became available, it was apparent that significant mismatches existed between *RV73BF* and several *B-FI* and *B-FIV* alleles including alleles found so far only in broiler chickens (Figure 1A). To include these alleles in PCR amplifications for SSCP, an additional reverse primer, *RV290BF*, was designed. *RV290BF* is directed toward a highly conserved region partially overlapping the priming site of *RV73BF*, but more 3′ to avoid a region of sequence variability seen in *B-FI* and broiler *B-F* sequences. The priming site for *RV290BF* is still within exon 2 except for the last nucleotide, a "T" within the 3′-intron. The SSCP patterns generated with the *OL283BF/RV290BF* primer pair allow us to distinguish the seven *B-F* alleles present in the MHC *B* congenic lines (Figure 2B) and seven *B* haplotypes identified in commercial broiler breeder lines (Figure 3A).

Design and Testing of B-L SSCP Primer Pairs

Alignments were made of sequences from Leghorn and broiler *B-Lβ* loci (Figure 1B), and the four criteria described above for *B-F* sequences were used for developing primer pairs for detecting sequence polymorphism in the chicken class II*β* genes. Primer pair *OL284BL/RV280BL* (Table 1) directed to sequences surrounding the hypervariable region within exon 2 of the *B-LBI* and *B-LBII* loci was found to be the most useful of six primers that were tested in various pair combinations. *OL284BL* crosses the intron/exon boundary with 15 of the 18 nucleotides corresponding to sequence within the upstream intron. This primer pair produces a PCR product of 277 bp and encompasses essentially the entire exon 2 (Figure 1B). Because of their larger size, optimal separation of *B-L* conformers was found to require longer electrophoresis (overnight runs at 70 V) (Figure 3B) although distinctive patterns can also be detected in shorter runs (Figure 2C). *OL284BL/RV280BL* reliably reveals the *B-L* haplotype SSCP patterns presented by 14 standard Leghorn and broiler haplotypes.

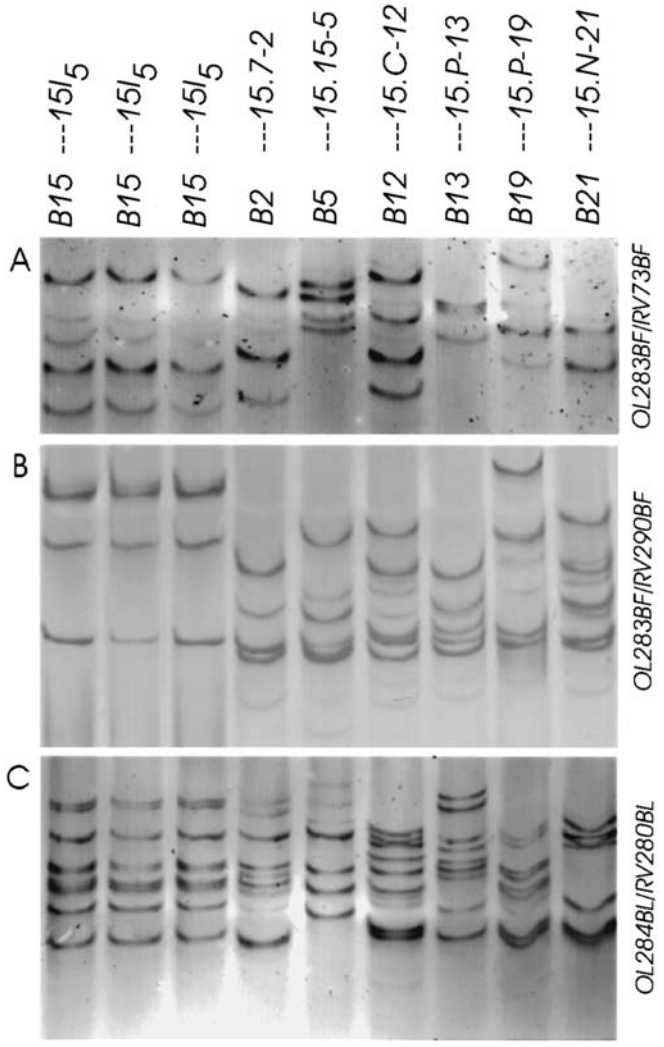

FIGURE 2. *B-F* (A and B) and *B-L* (C) single-strand conformation polymorphism (SSCP) patterns presented by the ADOL 15-*B* MHC congenic lines carrying seven different MHC *B* haplotypes (Lamont et al., 1990). Primer pairs are noted at the right. Lanes are identified with the *B* haplotype and name of source line. Patterns for three different individuals of the same homozygous genotype, B^{15}, are provided to illustrate the reproducibility of SSCP patterns.

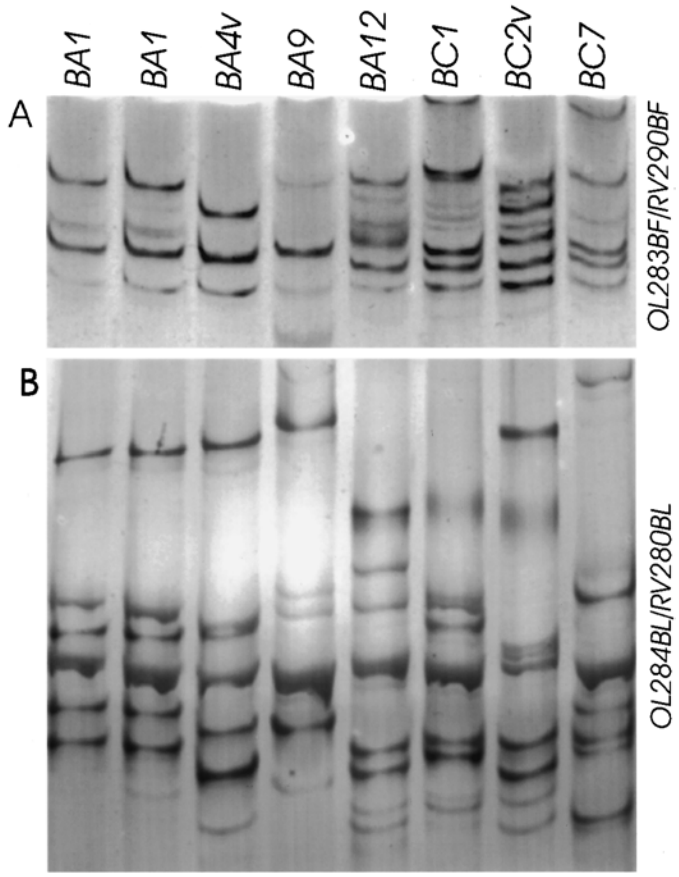

FIGURE 3. The *B-F* (A) and *B-L* (B) single-strand conformation polymorphism (SSCP) patterns for seven MHC *B* haplotypes (Li et al., 1999; Livant et al., 2001) from commercial broiler breeder lines. Primer pairs are noted at the right. Patterns for two different individuals of the same homozygous genotype, B^{A1}, illustrate the reproducibility of SSCP patterns. Note: Slight overloading of the samples has resulted in some distortion of the heaviest bands in the *B-L* SSCP patterns.

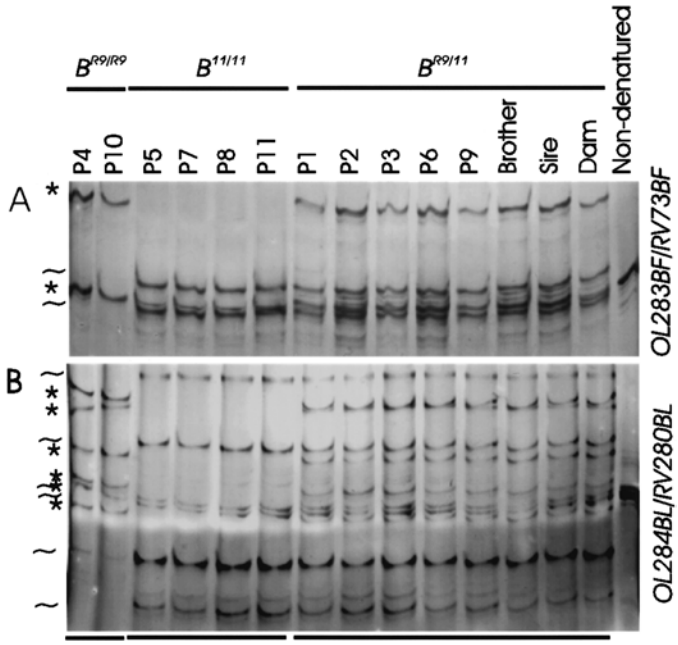

FIGURE 4. *B-F* (A) and *B-L* (B) single-strand conformation polymorphism patterns of the fully pedigreed C084 family (Briles et al., 1993) in which two *B* and three *Rfp-Y* haplotypes are segregating. Primer pairs are noted at the right. Samples were sorted according to previously defined *B* genotypes (Briles et al., 1993) prior to analysis. Bands originating from B^{R9} are marked with "*" and those from B^{11} with "~". Nondenatured control samples are provided to illustrate the size uniformity of the PCR product.

Evaluation of SSCP Typing with a Fully Pedigreed Family of Known B and Rfp-Y Haplotypes

To evaluate the ease with which segregating *B-F* and *B-L* haplotypes might be distinguished by SSCP, we first examined the SSCP patterns in a fully pedigreed family, C084, previously typed for *B* and *Rfp-Y* (Briles et al., 1993). The C084 family is composed of 11 progeny plus the sire, dam, and brother to the dam. As previously demonstrated by serology and Southern hybridization, two *B* and three *Rfp-Y* haplotypes segregate within this family (Briles et al., 1993). When the *B-F* and *B-L* SSCP patterns were sorted according to these previously determined *B* types, a perfect correspondence in SSCP pattern was found among the samples within each group (Figure 4). The two B^{R9}/B^{R9} individuals have identical *B-F* and *B-L* SSCP patterns, as do the four individuals homozygous for *B11*. The SSCP patterns for the B^{R9}/B^{11} heterozygotes are essentially identical to the patterns of B^{R9} and B^{11} combined. From this concordance we conclude that the primers for *B* class I and class IIβ alleles are specific and allow segregating *B* haplotypes to be identified.

Evaluation of SSCP Typing with DNA from 20 Randomly Selected Birds with Unknown B Genotypes

SSCP typing was further tested with DNA from birds of unknown *B* genotype to see how well SSCP patterns reveal *B-F* and *B-L* allelic differences in a population in which several *B* haplotypes are segregating. The DNA samples used for this test were from 20 randomly chosen individuals from Charles River SPAFAS line 22. At least four *B* haplotypes are known from serological typing to segregate within the SPAFAS line 22 with two haplotypes present at higher frequency, as noted by T. Girschick (2000, Charles River/SPAFAS, Storrs, CT; personal communication). The *B-L* and *B-F* SSCP patterns generated by *OL284BL/RV280BL* and *OL283BF/RV73BF*, respectively, and the *B-G* rfp for the 20 samples are presented in Figure 5. Unsorted patterns are on the left; patterns sorted into like categories are on the right. Seven *B-L* SSCP patterns were found among the 20 samples. Eight individuals were found to share one pattern (Category II), while other patterns were shared among fewer individuals. Single individuals represented two categories. The *B-F* SSCP patterns were similarly sorted. Nineteen of the 20 *B-F* patterns sorted into seven categories corresponding to the seven categories defined by *B-L* SSCP patterns. We expected this concordance given the close linkage between the *B-F* and *B-L* loci (Skjodt et al., 1985; Hala et al., 1988). However the pattern of Sample 3 did not meet this

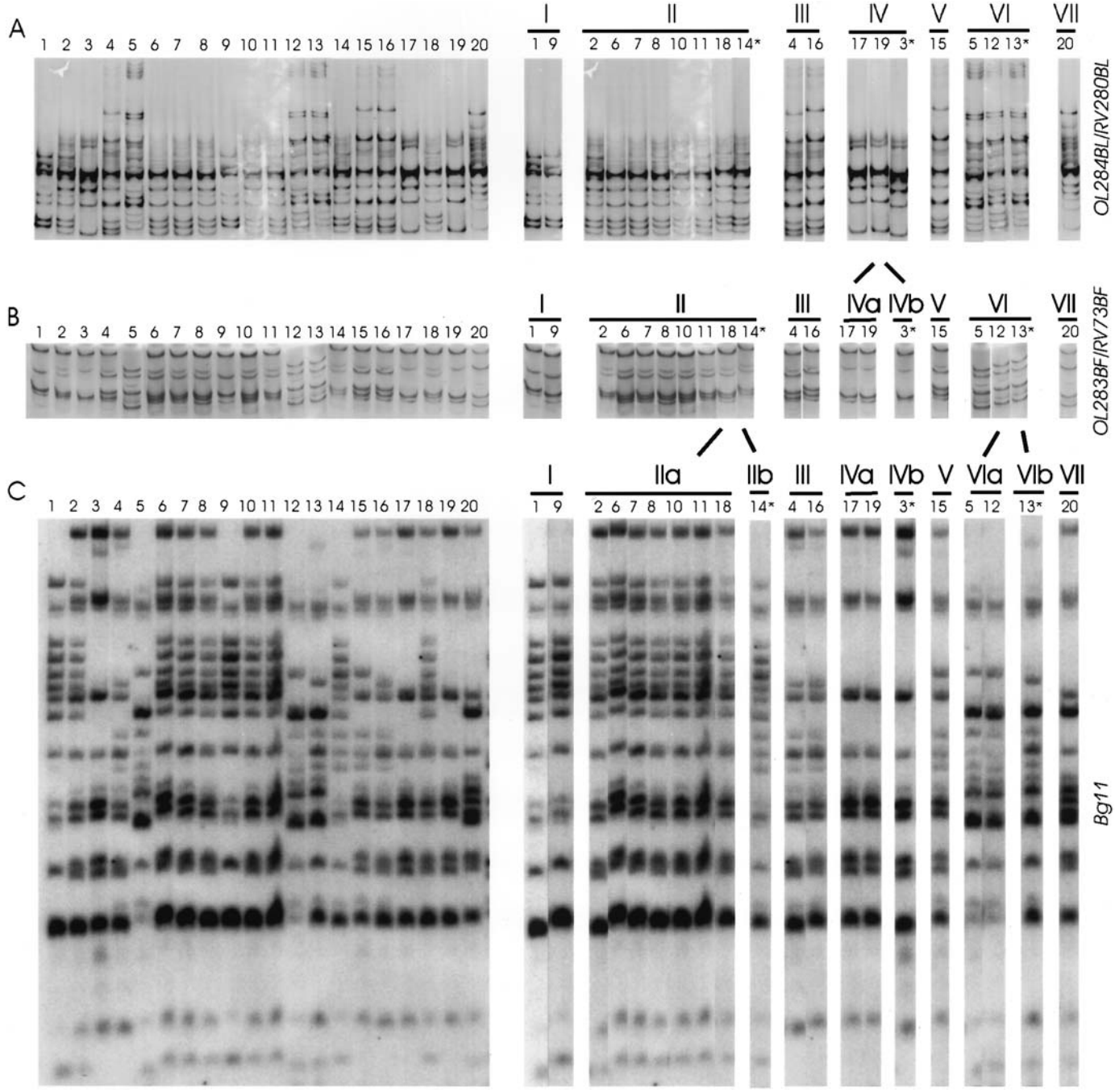

FIGURE 5. MHC *B* genotyping of 20 SPAFAS chickens using *B-L* single-strand conformation polymorphism (SSCP) (A), *B-F* SSCP (B), and *B-G* restriction fragment pattern (C). Patterns of the samples as loaded in numerical order are presented at left with the same samples sorted into like categories at the right. At the RIGHT the patterns have been sorted into like groups. Concordance was found among the *B-G*, *B-F*, and *B-L* patterns for each sample except for three samples that are denoted by asterisks and which may represent presence of recombinant haplotypes.

expectation. The *B-F* pattern of Sample 3 differs subtly (by the absence of a single band) from that of the other two members of Category IV. We therefore divided Category IV into two parts, IVa and IVb.

To further evaluate the *B* genotypes in SPAFAS line 22, we also genotyped the 20 samples by *B-G* rfp (Miller et al., 1988a). The *B-G* full-length cDNA clone *bg11* was used to probe DNA digested with *Pvu*II. There was generally a good match between *B-G* rfp-defined categories and those defined by *B-L* and *B-F* SSCP as expected because of the tight linkage that exists between the alleles at the loci within these three *B* subregions. Sample 3 is again different from Samples 17 and 19 supporting the earlier separation of this sample into a separate subcategory based on its *B-F* SSCP pattern. Two additional samples, Sample 14 and 13, also provided distinctive *B-G* rfp requiring them to be placed in subcategories, IIb and VIb, respectively, because of the presence of distinctive *B-G* alleles. Thus, when used in parallel with *B-G* rfp typing, *B-L* and *B-F* SSCP assays provide a sensitive means of

defining genetic variability within the *B* system at a resolution not available previously.

DISCUSSION

The tests presented in this report support the utility of SSCP in detecting *B* genotypes in both egg and broiler chickens. As illustrated by the 20 samples from SPAFAS line 22, the combination of SSCP typing for class I and class II alleles with *B-G* rfp provides a means by which *B* genotypes can be defined at high resolution. The existence of three discordant variants within the SPAFAS samples is an interesting finding. These could represent haplotypes derived at some point by recombination among haplotypes segregating within SPAFAS line 22. Whether these samples indeed represent recombinant haplotypes will require further investigation. There is clear evidence from the analysis of fully pedigreed families that recombinant *B* haplotypes occur periodically. Many of these appear to be the result of crossing over within the *B-G* region leading to new combinations of *B-G* restriction fragments (Briles et al., 1982a; Miller et al., 1988b; Goto et al., 1994; Livant et al., 2001). Further genetic variation apparently arises through intra-allelic exchange (Hunt et al., 1994). Such recombinant haplotypes are particularly valuable in determining the loci within the MHC that are responsible for the strong influences of the *B* system on immune response and disease resistance in chickens (Aeed et al., 1993; Schat et al., 1994; White et al., 1994; Golemboski et al., 1995).

The goal of this project was to develop practical DNA-based methods for comparing genetic differences at the *B* complex using simple laboratory equipment. The method was developed with an emphasis on obtaining information from the two *B-F* loci and the two *B-L* loci in single determinations. Typing is accomplished by comparing SSCP patterns representing haplotypes rather than individual bands. Hence, if individuals are heterozygous at both loci within the class, as many as four gene sequences can be present within the PCR reaction product. For simpler differences, such as single nucleotide substitutions distinguishing mutant and wild type alleles, conditions can be developed (using priming site choice, gel running conditions, temperature, etc.) for optimal separation of the single strands corresponding to the genetic differences. The genetic origin of individual products can then be easily assigned as noted earlier (Oto et al., 1993). In the more complex systems where there are more loci, such as the *B-F* or *B-L* haplotypes, often the individual bands can still be assigned to each haplotype. This can be seen in Figure 3 where two *B* haplotypes are segregating.

Because the MHC loci are highly polymorphic with the alleles typically differing from one another by several nucleotides, it is not possible to precisely optimize SSCP conditions for each allele. The conditions used in this study are conditions in which the individual haplotypes that we tested could be distinguished from each other. Therefore the patterns may be more complex than might be expected, e.g., band numbers fewer or greater than expected, and hence, an emphasis has been placed on tracking patterns rather than individual strands. Because the conditions are not optimized for each allele pair, the migration of single strands with similar conformations may not be fully resolved. In some instances, single-strands originating from different alleles may migrate essentially identically in the SSCP gels such that bands appear that are denser than others within a pattern. Again, since conditions cannot be precisely optimized for each allele, it is possible that identical strands from one allele may take more than one conformation such that these may contribute more than one band to the SSCP pattern. Further, while the initial conditions for the PCR reactions are highly stringent for priming only at the desired sites, minor, contaminating products may be obtained from irrelevant priming sites in reactions. Under some conditions these contaminating products may contribute to the SSCP pattern. With these qualifications in mind and with careful attention to detail, SSCP can be used to define *B* haplotypes. The analysis of large numbers of samples is greatly aided if the images are digitized and sorted as illustrated in Figure 5. Alternatively, incorporating automated detection of fluorescently-tagged PCR products could increase the throughput of *B* typing by the PCR SSCP and provide graphic data suitable for comparison.

Should *B* genotyping by SSCP patterns become widely adopted, it is likely that it will be difficult to relate genotypes defined in different laboratories without common standards. Given the sensitivity of SSCP to conditions under which gels are run, it is likely that SSCP patterns determined in different laboratories for the same *B* haplotype may appear somewhat different. For some purposes it may not be necessary to assign standard haplotype designations, but it certainly would be advantageous to have a means for establishing at least tentative standard *B* haplotype designations. Perhaps DNA representing the standard *B* haplotypes, such as those recently summarized (Miller et al., 2001), might serve as internal controls.

The primer sets described here are only a few of many that might be used in SSCP typing for the *B* system. The primers described here were designed to detect allelic polymorphism at the two *B-F* and two *B-L* loci with minimal effort. If desired, primer sets could be refined to be locus specific, and each locus could be assayed individually. Single-locus SSCP might be desirable under some circumstances since the resulting patterns of conformers would be simpler. Single-locus SSCP might suffice for routine typing in closed flocks where the haplotypes are well known because of the tight linkage among these four *B* loci. The SSCP assays described here detect sequence polymorphism only in the most polymorphic region of these loci. Additional genetic variability occurring outside these regions that might define additional *B-F* and *B-L* alleles would not be revealed in these assays, but could be encompassed by additional PCR-SSCP primer pairs.

SSCP typing may be generally valuable for detecting genetic diversity at other loci within the chicken MHC.

For example, we are utilizing *Y-F* class I SSCP assays to detect haplotypes within the *Rfp-Y* system (Briles et al., 1993; Miller et al., 1994; Afanassieff et al., 2001). Generally we find good correspondence between *Rfp-Y* haplotypes defined by class I restriction fragment patterns and *Y-F* SSCP patterns. SSCP typing is also useful in some instances for *B-G* genotyping. Simple *B-G* SSCP assays made with primer pairs designed to hybridize to highly conserved regions within the *B-G* genes produce large numbers of conformers that result in SSCP patterns that are difficult to interpret. Assaying subsets of the *B-G* gene family are more useful. For example, when SSCP assays are combined with a reverse transcriptase step to assay *B-G* transcripts relatively simple, distinctive SSCP patterns for particular haplotypes are obtained (Miller and Goto, 1993). Alternatively, PCR primer sets might be designed so that they hybridize only with subsets of the *B-G* gene family and result in simpler, interpretable SSCP patterns. SSCP might be adapted for genotyping additional polymorphic *B* loci, such as the TAP loci (Kaufman, 2000) as well. It is likely that at least some of the polymorphic loci within the MHC function coordinately (Kaufman, 2000). Broader application of SSCP assays may be an efficient means of defining allelic variability at polymorphic loci within recombinant haplotypes as efforts to understand the strong role of the *B* system in disease resistance continue.

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