

A single base insertion in *F9* causing hemophilia B in a family of Newfoundland-Parti Standard Poodle hybrid dogs.

Supplemental Table S1: Primer sequences used for DNA amplification and sequencing of canine *F9* exons and adjacent regions and primer sequences used for TaqMan® SNP Genotyping Assay for *F9* insertion.

Exon	Binding Site	Primer Sequence	Amplicon Size (bp)
1	5' UTR	F: 5'-TGTGTCACCTCCGGCTTCAG-3'	67
	Intron 1	R: 5'-TCTACAGCTAGAAGACAAGCATAC-3'	
2	Intron 1	F: 5'-TTGAATTTTCAGTTTTTCTTGATCG-3'	164
	Intron 2	R: 5'-TGCATCTGAAGAACATTACGC-3'	
3	Intron 2	F: 5'-GCAGTTTTGAAGAAGCACGGG-3'	25
	Intron 3	R: 5'-ACACAGAGAAAAGATACCTAATTCTCA-3'	
4	Intron 3	F: 5'-AAGACAGGGGCATCCCATATC-3'	114
	Intron 4	R: 5'-CTTCCATTAAGTGTTCTTACCAC-3'	
5	Intron 4	F: 5'-CTCCCAAGCCTCTTTCCATG-3'	129
	Intron 5	R: 5'-CAAACAGGGTTTATGAAAGTATGTGAAC-3'	
6	Intron 5	F: 5'-CGTTCCGCTTGCCAATGAAAAATA-3'	197
	Intron 6	R: 5'-CTCTGGGCTCCAGTTTTTGAC-3'	
7	Intron 6	F: 5'-ACACCCCTGCCTATCAACAG-3'	115
	Intron 7	R: 5'-GCTCCTCTAGCATTAGCCC-3'	
8	Intron 7	F: 5'-CCTTGGCAAATACGTTTATGTGTAAG-3'	548
	3' UTR	R: 5'-TGCCAAGGAGGGGAGAATC-3'	
TaqMan® SNP Genotyping Assay			
8	Intron 7	F: 5'-TGTAAGTCTACCTGTGACTGAAGAGAA-3'	90
	3' UTR	R: 5'-CGTTTCGCTTCTGCTCTGTATGT-3'	
	VICTM	5'-TCTTCTCGGTGTTATGC-3'	
	FAMTM	5'-TTCTCGGTGTTTATGC-3'	

bp, base pairs; F, forward primer; R, reverse Primer; UTR, untranslated region; VIC™, Dye label at 5' end; FAM™, Dye label at 5' end (Thermo Fisher Scientific)

Standard PCR protocol was used: 1) Pre- Denaturation (95°C, 5 min); 2) Denaturation (95°C, 0.5 min); 3) Annealing (59.5°C, 0.5 min); 4) Elongation (72°C, 1 min); 5) Denaturation 95°C, 45 sec); 6) Annealing (59.5°C, 45 sec); 7) Elongation (72°C, 45 sec); 8) Final elongation (72°C, 10 min); and 9) Cooling (4°C, ~2 hours). Step 2-4 (9 cycles), and step 5-7 (30 cycles).

