

The first report of a Ciconiiformes defensin cluster: Characterization of β -defensin genes in the crested ibis based on BAC libraries

Hong Lan[†], Hui Chen[†], Li-Cheng Chen[†], Bei-Bing Wang, Li Sun, Mei-Ying Ma, Sheng-Guo Fang, and Qiu-Hong Wan*

Supplementary Table S1. Summary of average GC content of duplicated and non-duplicated regions in four birds.

Species	Duplicated region			Non-duplicated region
	<i>AvBD1/AvBD3</i> subfamily	<i>AvBD7</i> subfamily	tRNA cluster	
Chicken	51.79% ($\chi^2=67.463$, $P=0.000$)	49.05% ($\chi^2=11.828$, $P=0.001$)	51.75% ($\chi^2=20.766$, $P=0.000$)	46.48%
Duck	54.62% ($\chi^2=587.862$, $P=0.000$)	/	55.82 % ($\chi^2=218.481$, $P=0.000$)	45.41%
Zebra finch	53.36% ($\chi^2=761.381$, $P=0.000$)	/	48.04% ($\chi^2=27.613$, $P=0.000$)	45.30%
Crested ibis	51.78% ($\chi^2=183.726$, $P=0.000$)	/	52.73% ($\chi^2=177.313$, $P=0.000$)	45.13%

The Pearson's chi-squared test was performed for the average GC content of each duplicated region, and relative results are given in brackets (all $df = 1$). The boundaries of each region are shown in Figure 3 with dotted lines.

Supplementary Table S2. Gene-specific primers for 3' and 5' RACE of crested ibis defensins.

Primer name	Primer sequence (5' to 3')	Location	Size (bp)	Ta (°C)
AvBD1 α F2	F:GCACTACTGGTTTGCCAATTTCCCCTTA	<i>AvBD1α</i>	319	70.5
AvBD1 α R1	R:CAATGGCAATAAGGGGAAATTGGCAA		313	71.0
AvBD1 β F2	F:AGGGGGATTCTGTAGTTTTTTGAAATGCTA	<i>AvBD1β</i>	327	68.0
AvBD1 β R1	R:TGCAGCAAACCATAAATGTTGAACACCTT		296	70.0
AvBD2F1	F:TCTCCTCTTCTTGGCACTCCAGGTTTC	<i>AvBD2</i>	384	69.0
AvBD2R1	R:AACCCAAAGCAACTTCCAACCTTAACCAGAT		250	70.5
AvBD3F1	F:CCTGCTCTTCCCCTTCTTCCTCCTGTT	<i>AvBD3</i>	399	71.0
AvBD3R1	R:CAGGGTAGCGGCAGTCCCCAAAACCT		231	72.0
AvBD4F1	F:TCCTCTTGGTGGTACTTCACGGAGCTG	<i>AvBD4</i>	329	70.5
AvBD4R1	R:GGGCACATCCCAGGGAAGCAGAAAGTC		259	74.5
AvBD5F1	F:TCCTCTGTGCTTTCCTCCTCCTAATGC	<i>AvBD5</i>	394	69.0
AvBD5R1	R:GCGGCGTTCACAGTCCTGGGGCAAT		355/161	77.0
AvBD7F1	F:GTTGTCATCTTCATGATGCTCCAAGGGG	<i>AvBD7</i>	406	71.5
AvBD7R1	R:TCGACGACATATCCCTGGGAAGCAGT		218	71.0
AvBD8F1	F:CTTTCCCTTTTCTTTGCTGTTCTCCTCTT	<i>AvBD8</i>	738	69.0
AvBD8R1	R:CCGAAGGACCTCGTATTGGGTAGATTACAG		223	70.5
AvBD9F1	F:CTCTTCTTCCTCTTCCAGGCTGCTCC	<i>AvBD9</i>	394	69.0
AvBD9R1	R:GGTCCCAATGTCAACCAGAGGAGAAGTG		230	71.0
AvBD10F1	F:ATCCTGTACCTGCTCTTTGCTGTTCTCCTC	<i>AvBD10</i>	566	70.5
AvBD10R1	R:ACGACCCCGAGGCAGCGAAGGTG		235	75.0
AvBD12F1 ^a	F:CCTTTGGTTCATTTTAATCTTCATCTCCCTG	<i>AvBD12</i>	513	70.0
AvBD12F3 ^a	F:TGGTTCATTTTAATCTTCATCTCCCTGA		509	66.0
AvBD12R1	R:ACAGCAGAGAATGATGGGTCAAAGCAG		260	70.0
eAvBD13F1	F:GCAGTCATTGCCATTCTCCTCCTCCAG	<i>AvBD13</i>	301	72.0
eAvBD13R1	R:GCCGTGGTTGCTTCTGCACTGCTGGCT		158	78.0

^a nest-PCR was applied in 3' RACE of *AvBD12*.

Supplementary Table S3. Primers used to fill the gaps in the duck defensin cluster.

Primer name	Primer sequence (5´ to 3´)	Fragment	Size (bp) ^a	Ta (°C)
AvBD3gapAB-F1	F:CAGCACATCTCCCTCCTTCC	<i>AvBD3A</i> –	2927	55.0
AvBD3gapAB-R2	R:GGCTGCCAGTTCGCTGAAAAAC	<i>AvBD3B</i>		
AvBD3gapB-F1	F:GAAGAGCAGGAACAGGATC	<i>AvBD3B</i>	1547	53.0
AvBD3gapB-R1	R:CTCCCATGCTGTGAGTTA			
AvBD3gapBE1-F2	F:CTCACAGCAATGGGAGAGC	<i>AvBD3B</i> –	1040	56.0
AvBD3gapBE1-R2	R:CAGACAAAGAGGGGGAATC	<i>AvBD3C</i>		
AvBD3gapBE2-F1	F:ATCTCCCAATAGGTGTC	<i>AvBD3C</i> –	3292	50.0
AvBD3gapBE2-R2	R:GGTAAGGTCTGGGTTC	<i>AvBD3D</i>		
AvBD3gapBE3-F4	F:TAACAAGGAAGCAGAAA	<i>AvBD3D</i> –	3317	50.5
AvBD3gapBE3-R1	R:CTGTTTGCTGCGTGTGA	<i>AvBD3E</i>		
AvBD3gapEF-F2	F:CTGATGGAGGTCCTGGTGTC	<i>AvBD3E</i> –	1680	55.0
AvBD3gapEF-R1	R:AGAGGCACCATCACAAAGG	<i>AvBD3F</i>		

^a In view of unknown gap sizes expected, all PCRs adopted extension time as 3 min.