

Gene symbol	<i>Flcn</i>			
primer	Sequence(5'-3')	Size	target gene	accession
BHDRI BHDRJ	CCCTCTGCCACTTCTGCGA CTCGCACATGTCTGACTTCT	231bp	<i>Flcn(Bhd)</i>	NM_199390.2
comment	BHD Rats have a 1-bp insertion at exon 3 of <i>Flcn</i> ( <i>Bhd</i> ) gene on rat chromosome 10. Genotyping by sequencing analysis.			

PCR condition							
Taq polymerase		BIOTAQ™ HS DNA Polymerase (Bioline Reagents Ltd., UK)					
Thermal cycler		PC-808(ASTEC)					
PCR buffer		Ampdirect Plus					
		<b>first denature</b>	<b>denature</b>	<b>anneal</b>	<b>extension</b>	<b>cycle</b>	<b>final extension</b>
PCR	°C	94	94	60	72	35	72
	min	3	0.5	1	0.75		3
comment		PCR method; Blood was applied to FTA®card (GE Healthcare UK Ltd.,UK) and dried. 1.5mm FTA disc was removed from the bloody-stained region on FTA®card. Untreated sample discs were placed directly in 15 µL PCR mixture containing 1 × Ampdirect®Plus, 0.2 µM each primer and 0.4 units of BIOTAQ™ HS DNA Polymerase.					

Sequenece: ABI3130x/	
Wild type allele	<p>G T G C G G G G G C C A T G G A</p>
Heterozygote (BHD/Dspe)	<p>G T G C G G G G G G C C A A T G G</p> <p style="text-align: center;">↑ 1bp insertion</p>
Strain harboring mutation	BHD/Dspe