

PCR Reaction Solution (Total volume for one reaction: 20ul):

1.5ul tail preps DNA
2ul 10X PCR buffer (without MgCl₂)
2ul 25mM MgCl₂
1ul 2.5mM dNTPs
1ul 20uM Forward Primer
1ul 20uM Reverse Primer
0.1ul Taq enzyme (5U/ul)
11.4ul dH₂O

PCR protocols:

- For Col4A3 Mut (KO) and Col4A3 WT
- S1: 94C 1min 30sec
S2: 94C 30sec
S3: 65C 1min 30sec
S4: Go to S2, Repeat 32 times
S5: 4C Hold

Col4A3 Mut

Neo1F – CTT GGG TGG AGA GGC TAT TC

Neo2R – AGG TGA GAT GAC AGG AGA TC

Col4A3 WT

	KO	Het	WT
Col4A3 Mut (280bp)	+	+	-
Col4A3 WT (300bp)	-	+	+

Col4A3WT1F – AAC ACC AGC TCT GAT GCC AAT G

Col4A3WT2R – AAT GAA AGA AAA ACC TTT CCA GAG

- For Human Col4A4 (Tg)
S1: 94C 2min
S2: 94C 1min
S3: 50C 1min
S4: 72C 30sec
S5: Go to S2, Repeat 30 times
S6: 72C 30sec
S7: 4C Hold

Col4A4 (exon 48)

Col4A41F – GTG TGT GTC TGA GCC CTA AT

Col4A42R – TGG TGA ATT TCG CAT TCT

	Tg	Het	WT
Col4A4 (322bp)	+	+	-

Analysis of amplified samples: 1% agarose gel