

primer	sequence 5'-->3'	primer type
Forward common	CTCTGCTGCCTCCTGGCTTCT	Forward common
Wild type reverse	CGAGGCGGATCACAAGCAATA	Wild type
CAG promoter reverse	TCAATGGGCGGGGTCGTT	mutant

amplicons: 0.32kb for wild-type, 0.25kb for EGFP-L10a knock-in.

Reactions/components	Volume (µl)	Final Concentration
ddH2O	8.2	-
2 X GoTaq Master Mix (Pror	10	1 X
Forward common	0.4	0.2 uM
Wild type reverse	0.4	0.2 uM
CAG promoter reverse	0.6	0.3 uM
DNA	0.5	-

Cycling Step #	Temp °C	Time	Note
1		94 5 min	-
2		94 30 sec	-
3		58 30 sec	-
4		72 30 sec	repeat steps 2-4 for 35 cycles
5		72 5 min	-
6		10 -	hold

Note

The 0.25 kb band is usually weaker than the 0.33 kb one when using a mix of these three oligos at 1:1:1 ratio, likely due to high GC content in CAG promoter. Somehow adding 20-50% more of CAG promoter reverse oligo gives comparable two bands.