

GENOTYPING BY PCR PROTOCOL

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NAME OF PCR: BTBR.B6-Qk^{qk-k2}/Mmucd MMRRC # 000056-UCD

Protocol:

Reagent/ Constituent	Volume (μL)
Water	5.6
10x Buffer (contains 15mM MgCl ₂)	2.0
dNTPs (stock concentration is 1.25mM)	4.0
Primer 1(stock concentration is 10μM) qk-k2 F	0.5
Primer 2 (stock concentration is 10μM) qk-k2 R	0.5
FastStart Taq 5U/μL	0.2
DNA Sample	1.0
TOTAL VOLUME OF REACTION:	12.8μL

Comments on protocol:

- PCR followed by restriction digest with *DdeI*. Assay takes advantage of single base pair change (*qk*^{k2} mutation) in exon 4 (GenBank AJ012814) that creates a unique restriction enzyme recognition site. This assay allows all genotypes (wild type, homozygous and heterozygous) to be distinguished. Note: homozygotes (BTBR.B6-Qk^{qk-k2}/Mmcd) are not viable and die at e9.5-11.5.

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	94	5:00	1
2. Denaturation	94	0:30	} 35x
3. Annealing } steps 2-3-4 will cycle in sequence	59	0:30	
4. Elongation	72	0:30	
5. Amplification	72	7:00	1
6. Finish	4	∞	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1: qk-k2 F	GAGGAGCAAAATAGAGGCAAGC
2: QK-K2.R	CCACATCACTTCCTTCCACAAAA
sequence with QK-K2.R primer	

Electrophoresis Protocol:

Agarose: 1.5% V: 90 Estimated Running Time: 90 min.

Expected Bands	Genotype
77 bp and 275 bp	WT
12 bp, 65 bp, 77 bp and 275 bp	Het
12 bp, 65 bp and 275 bp)	Hom
SNP found at position ~ 240 bp of sequencing	

Mutation site (red) and flanking sequence:

WT: aatcactgTggaagatgctc

Mutant: catcttccAagtgattagt

T = wild-type base (mutant has "A")

ttcttttaagaggagcaaaatagaggcaagccaattgggagcatctaataagacttacatgtactaatcactgTggaagatgctcagaacagagcagaaatcaagctgaa
gagagcggtgaagaagtgaagaagtactgtacctgcggttaagtaaatctcatctgaattttttaccttttagattgactgaaattgtaattgtaataaagtatgattgtatc
tgtagagatttttaaccacaaataaaaaaatgtgtggctcattaagtatatagtgtaaaaagacaggattgctcaaaaatccctgctattttgtggaagggaagtgtgttag

Reverse Complement: Sequenced with R Primer around 240bp is the snp (A=wt, T=mut)

Ctaccacatcacttcctccacaaaaataagcaggatttttgagcaatcctgtcttttactatataactaatgagccacacattattttatattgtggtaataaaatctctaacagata
acaactcatactttattaacattacaattcaagtcaatctaaaaaggaaaaggtaaaaaaaatcaagatgaaaatttactaccgcaggatccagtaacttctcacttctcaaccgc
tctctcagctgtatttctgtctgttctgagcatctccAagtgattagtacatgtaagcttcatttagatgctcccaattgggtgctctattttgctcctctttaaagaa