Masako's Capn4 PCR Genotyping Protocol

Primer(1): for- 5'-GTCAGGCTAGATGCCATGTTCC
Primer(2): rev-5'-GTTCACTTGGATCTGTCCGGTGCC
Primer(3): rev-5'-CGACTATCCGAGCGCTGCC
Capn4-r452: CAAGATGGAGCTGGAGAGAT

Primer (1) and Primer (2) are located within exon 9 and 10, respectively. Primer (3) is located within the deleted intron 9 from the Capn4 floxed allele.

When primers (1) and (2) are used, wild-type and floxed alleles give you a ~2k bp and a 373 bp bands, respectively. In theory, we should detect both bands in heterozygotes, but we only detect the floxed allele; probably because smaller fragment is more efficiently amplified under the conditions used.
When primers (1) and (3) are used, only the wild-type allele is amplified and that gives a ~1.2 k bp band(Primer 3 does not work fine).
When Primers (1) and Capn4-r452 are used, only the wild-type allele is amplified and that gives a ~500bp band. (Fig.1)
PCR thermal cycle conditions:

CAPN4 flox
95 degrees for 5min
95 for 1 min
64 for 1 min
72 for 40 sec
x 40 cycles
72 for 7 min

CAPN4 wt
95 degrees for 5min
95 for 1 min
60 for 1 min
72 for 40 sec
x 40 cycles
72 for 7 min

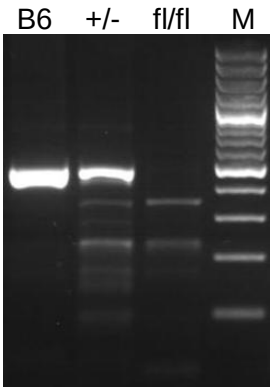


Fig.1

Fig.1

GTCAGGCTAGATGCCATGTTCC

Exon 9 CAPN4

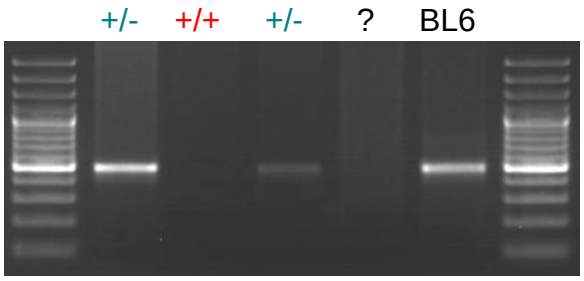
GATTCCACCTGAATGAGCATCTCTACAGCATGATCATCCGGCGCTACGCAGATGAGAGCG
GGAACATGGATTTCGATAACTTCATCAGCTGCTTGGTCAGGCTAGATGCCATGTTCC

Intron 9 CAPN4

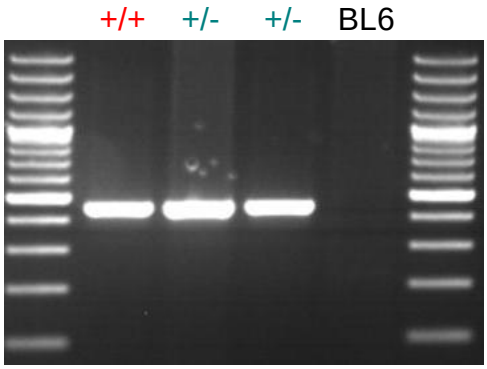
GTGAGTGTCACTCTAGATCTCTGCGAGGGTGGGGATTTGCTAACCTTCATTGGCCAAGGG
CTCCTCTAGGTCTAACCTTAATGTCAGCAATGCTGGGCTCACTAGGCAGGGACAGCTGAG
ACTTGTATGGCTGAGATTCTTTTTTTTTTTTTTTTTTTTTTTTTTTTAAAGATTTATTTA
TTACATGTGAGTACACTGTAGCTGTCTTCAGACACACCAGAAGAGGGCATCAGATCTCTT
TACGGATGGTTGTGAGCCACCATGTGGTTGCTGGGATTTGAACTCTGGACCTTTGGAAGA
GCAGTCGGGTGCTCTTACCCACTGAGCCATCTCACCAGCCCCTGGCTGAGATTCTTGTAT
GGCCATGTGGATACTGGGAATTGAACCCCAGGATCCTCTGCAAGAGCAGTAGGTGCTCTT
AACCTCTGAGCCATCTCTCCAGCTCCATCTTGCCTGGTTTCTTTTTCTTTTTTTTTTTTT
AAAGATTTATTTATTTATTATATGTAAGTACACTGTAGCTGTCTTCAGACACACCAGAAG
AGGGAGTCAGATCTTGTTACGGATGGCTGTGAGCCACCATGTGGTTGCTGGGATTTTGAA
CTCAGGACTTTCATAAGAGCAGTCGGGTGCTCTTACCTGCTGAGCCATCTCACCAGCCCC
TTGCCTGGTTTCTTGAGACACGTTTTCATGTAGTTCGGGTTGATTTGAGACTTGATTTGT
AGCTGAGAGTGACCTTGAACTTTTGACTCTGAGGTGAGAGCTTTTATTAGGATTGATGTA
AGACTACCTCCAAAATGGGAACTATTGTCCTCCTGCTAGGGTGGAACCCAACACTCTCCC
GAGCCAAGCACTTGCTCCTAGCTGCTACTCAACCAGGAAGATGGAAAGTCAGAATTTGAT
TCTGTGACTGCCTGAGAACTTAATTGAGCAATGAGAAAAAACAGGAGTGATGGTCAGGGG
TGTACTGTGCAGTGCTGTGGATATCAAGTTCAGGGGCAGCGCTCGGATAGTCGTGGGCGT
TGTAGAGCCAAGATAGCAGTTTCTAGGCAAGAAAGGGGCAGAGTCAGCCATGGCTTACAA
AGCTCCTTTGATCTGGCAGCCAAGGGAAGAGTGAAAACAGACGTTGGGTGTGGTGGTGCA
CATCTGTGACCCCAGCACTTAAAAAAGCTGAGGCCACAGGATGATCGGTTGTTCAAGGTC
ATCATTGACTACATCTCAGACCAAGATTAGTTCATTAGTTACTTAGTTAAAAGGGCTAGC
ATCTAGCTTAGTTGGGAGAGTGCTTGCCTCGCATACGTGAAGCCCTGGGTTTGTGACCAG
GCACTCATTAACCTGAATACGACAGACCTGTAATCCAGCAAGCAGGAGATAGAAGCAGGA
GGATAACAAGTTTAAGGAGAAAGGTCCTCTCACCTGTGGTAGCCAGGGCACCAGTAGCAC
CAAGGACACCATATGTGGCCACCTGAAGGTGGTGGTGACTCAGCCTTGGTACAGGAAGGA
ACAGATGTAGTGTGGGCAACGCTGGCTTGGTTTGGTTGTGGACAGAAGGCATGGATTCAA
GTTCCAGGGCAGGAGTGCGGACCACACTAGACGCTAAGGCCAAGATTGGCAAGTTACCTC
CTTTTAGGGGCCTGTTTTGGGTTACAGCTGGGAGGTTGTAGCATAGGTGTCAGGAAGGAT
GGGCCTGGGTTCCAGTCAGTTTTCTGTCCTGTCTGTCTGTCGTGTGATCTGCAACATATC
ACTTTGCCTGTTTATTTATTGAGATGTCAGGGATTGAACCCCATGCCTCAAGAATAATGG
GTGTATACTCTTTTACTGTGTTATAATACCTGAATTCTGTGCCTCATTTAAAAATGCCAC
ATGCTCACAGGATTAGACATTTTATTGATTTAACTTCCTCTTCGTCCTCCTCTTCCTCCT
CCTCCTCCTAATATTATTAATAGAGTCTCATGTAGCCTGACTAGCCTTGAACTCCCACAT
GTACCTGAAGGTAATAGTGAACTCAAGATCCTCCTACCATAACTCACCTAGTGCTGGGAT
CACAAGTGTGTATCATCACCACGTGTCTTCGAAGTTTTGTGGAGCGAGCGCTAACTTCCA
TCAACAACATCAACATGCTATAGCCAGTCAGGGTGGCCGTGCTTTTGATCCCAGCACTCT
GGAGGCAGAAGCAGGGAGCTCTCCATAAGTCTGAGGTCAACCAAAGGGTATTTAATGAGA
CCCTATCTAAAAACAAAGCAGAATAAAACAACAACATCAACAAGAGGGAAATAATTATAT
TATCGTTCTAGAAGCACCCAAGCCCTTCCTTTCTCCCCGCAG

CGACTATCCGAGCGCTGCC

CAPN4Z wt PCR:



CAPN4Z flox PCR:



Cre (generic)	PCR1: Cre (generic)	PV 83	GTC CAA TTT ACT GAC CGT ACA CC
		PV 85	GTT ATT CGG ATC ATC AGC TAC ACC