Prepared by Isabel Novoa

GADD34 KO1 (EX) genotype by PCR (10/01)

7S is a primer flanking the deletion on its 5' end.
6AS is a primer in the deleted segment
4AS is a primer flanking the deletion on its 3' end

Buffer: 1.5mM $MgCl_2$ (Fisher) or homemade 10x buffer (100mM Tris pH 8.3, 500mM KCl, 15mM $MgCl_2$)

Primers:

mMYD116.7S (2x) 5' CCA GGA GAG AAG ACC AAG GGA CGT G 3'

mMYD116.6AS (1x) 5' GGA GAT TGC AAG AGA GTG AAC ACA GC 3'

mMYD116.4AS (1x) 5' AA GCC TTC GCC ATC TGC TTA TCC AG 3'

PCR Products:

467 bp fragment for WT with	mMYD116.7S vs. mMYD116.6AS
~550 bp fragment for KO1EX (**mut allele**) with	mMYD116.7S vs. mMYD116.4AS
~1621 bp fragment for WT with	mMYD116.7S vs. mMYD116.4AS*

* this fragment may not always be obvious

PCR Conditions:

94˚C, 4 min.

(94˚C, 1 min; 64˚C, 1 min; 72˚C, 1.5 min.) x 35 cycles

72˚C, 10 min

4˚C, 30 min

RT

Comment:
This PCR assay works well on high quality genomic DNA (i.e proteinase K digested, phenol-chloroform extracted material) but does not work well on cruder preparations of mouse tail DNA (which do work in other PCR-based genotyping protocols)