STRAIN: EM12214
B6D2F1 x B6-Cdkn1a$^{em2(IMPC)Cnrm}$/Cnrm (synonym: P21 Knock-out)

CRISPR/Cas-mediated exon deletion:
Cdkn1a critical exon 2 is deleted, following CRISPR/Cas-mediated, targeted deletion

PRIMER P21 FOR **5'** AAACAGAGGCTGGAGACATGGAGT **3'**

PRIMER P21 REW **5'** CCAATCTGCGCTTGGAGTGATAGA **3'**

PRIMER P21 FOR 15pm / total volume 30ul Tm: 72°C
PRIMER P21 REW 15pm / total volume 30ul Tm: 72°C

PCR MIX

DNTPS (2mM)		3ul
PCR BUFFER 10x		3ul
PRIMER P21 FOR	(0.5pm/ul)	
PRIMER P21 REW	(0.5pm/ul)	
H2O		
TAQ POLYMERASE (5U/ul)		0.3ul
DNA		2ul
TOTAL		30ul

PCR PROGRAM:

1) 95°C x 2'00''
2) 95°C x 0'30''
3) 58°C x 0'30''
4) 72°C x 1'00''
5) step 2 to step 5 x 50 repeats
6) 72°C x 10'00''
7) 4°C forever
8) End

MUTATION: 414 bp

Background strains:

Zygote donor strain: 50% C57BL/6N + 50% DBA/2 (B6D2F1)

Backcrossing strain: C57BL/6N

F2 mice are ca. 87.5% C57BL/6N + 12.5% DBA/2

Mice are heterozygous for the Cdkn1a$^{em2(IMPC)Cnrm}$ mutation

The mutation was confirmed by locus sequencing

Method used to produce mutant mice:

CRISPR/Cas9 zygote injection with 2 sgRNAs to delete exon 2

Genomic location of deletion: chr_17:29098083-29099251

http://www.ensembl.org/Mus_musculus/Location/View?r=17:29098024-29099309