

**STRAIN: EM12215**

**B6D2F1 x B6-Cdkn1a<sup>em1(IMPC)<sup>Cnrm</sup>/Cnrm</sup>** (synonym: P21 Conditional)

CRISPR/Cas-mediated conditional mutation:

Cdkn1a critical exon 2 is flanked by two loxP sites, following their CRISPR/Cas-mediated, targeted insertion

**(PCR 1 - 5' LoxP site)**

**PRIMER 1**            **5' AACTCAGAAATCCGCCTGCCTCTG 3'**

**PRIMER 2**            **5' GTGTGAACATCCCAAATACGTGTG 3'**

**PRIMER 1**            15pm / total volume 30ul            Tm: 74°C

**PRIMER 2**            15pm / total volume 30ul            Tm: 70°C

**PCR MIX**

DNTPS (2mM)	3ul
PCR BUFFER 10x	3ul
<b>PRIMER 1</b>	(0.5pm/ul)
<b>PRIMER 2</b>	(0.5pm/ul)
H2O	
TAQ POLYMERASE (5U/ul)	0.3ul
DNA	2ul
<b>TOTAL</b>	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:** 210 bp

**WT:** 170 bp

**STRAIN: EM12215 (PCR 2 - 3' LoxP site)**

**PRIMER 3**                      **5' CCTATAACTAACTCTGGGCGTTGG 3'**

**PRIMER 5**                      **5' CCAATCTGCGCTTGGAGTGATAGA3'**

**PRIMER 3**                      15pm / total volume 30ul                      Tm: 76°C

**PRIMER 5**                      15pm / total volume 30ul                      Tm: 72°C

**PCR MIX**

DNTPS (2mM)	3ul
PCR BUFFER 10x	3ul
<b>PRIMER 3</b>	(0.5pm/ul)
<b>PRIMER 5</b>	(0.5pm/ul)
H2O	
TAQ POLYMERASE (5U/ul)	0.3ul
DNA	2ul
<b>TOTAL</b>	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:** 247 bp                      **WT:** 207 bp

**Background strains:**

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

Backcrossing strain: C57BL/6N

F3 mice are ca. 93.7% C57BL/6N + 6.3% DBA/2

**Mice are heterozygous for the  $Cdkn1a^{em1(IMPC)Cnrm}$  mutation**

**Method used to produce mutant mice:**

CRISPR/Cas9 zygote injection with 2 sgRNAs to insert 2 loxP sites, flanking in *cis* the critical exon 2