



**CNB-CSIC Mouse Embryo Cryopreservation Facility – Spanish EMMA node**

**GENOTYPING PROTOCOL**

EMMA ID number	<b>EM:05945</b>
Mouse mutant strain	<b>B6.129-Krastm1Bbd/Cnbc</b>
Gene/Locus affected	<b>Kras</b>
MGI ID number	<b>MGI:96680</b>
Mouse strain common name	<b>Krastm1Bbd</b>
Owner/Provider of this mouse strain	<b>Mariano Barbacid</b>
DNA primers for wild-type allele	5I0: 5'-AGGGTAGGTGTTGGGATAGC-3' 3Ex1: 5'-CTCAGTCATTTTCAGCAGGC-3'
PCR conditions for wild-type allele	Program: 5'94°;30x(1'94°,1'60°,1'72°);10'72° PCR product: 403 bp
DNA primers for mutant allele	5I0: 5'-AGGGTAGGTGTTGGGATAGC-3' 103rev-2: 5'-CTGCTCTTTACTGAAGGCTC-3'
PCR conditions for mutant allele	Program: 2'94°;30x(1'94°,30"63°,30"72°);10'72° PCR product: 621 bp
Source of genotyping information	<b>Provider or Pub ID: 20609353, 12957286, 16959882, 17349585, 21514245, 21665147</b>
Comments	<b>Primers 5I0: 5'-AGGGTAGGTGTTGGGATAGC-3', 3Ex1: 5'-CTCAGTCATTTTCAGCAGGC-3'), and 103rev-2: 5'-CTGCTCTTTACTGAAGGCTC-3'). PCR products are 403 bp (5I0/3Ex1) for the wt allele and 621 bp (5I0/103rev-2) for the targeted K-ras allele. In tissues and MEFs in which the floxed STOP cassette present in the targeted K-ras allele allele has been excised, primers 5I0 and 3Ex1 yield a DNA fragment of 669 bp.</b>