PCR genotyping protocol for Gcg-RSR-CreER^{T2} mice

Gcg-RSR-CreER^{T2} allele can be screened by standard PCR genotyping protocol using generic Cre primers or Gcg-CreER^{T2}-specific primer set.

Generic Cre primer set

Cre685	upstream	5'-ACCTGAAGATGTTCGCGATTATCT	Amplicon 370 bp
Cre1054	downstream	5'-ACCGTCAGTACGTGAGATATCTT	

Gcg-CreERT2-specific primer set

GcgS1	upstream	5'-AACATGGCATTGGAGCCATAAGCA	Amplicon 398 bp
Cre685A	downstream	5'-AGATAATCGCGAACATCTTCAGGT	

PCR cycling

Step	Temperature	Time	
Initial denaturation	94°C	3 min	1 cycle
Denaturation	94°C	1 min	
Annealing	60°C	30 sec	35 cycles
Extension	72°C	20 sec	
Final extension	72°C	7 min	1 cycle

Detection of Gcg wild-type allele to determine zygosity.

Gcg primer set

GcgS1	upstream	5'-AACATGGCATTGGAGCCATAAGCA
GcgA2	downstream	5'-GTATATGTATCCTGATTCGTATCCCA

Amplicon size
Wild type: 403 bp
Mutant: 3315 bp

The PCR protocol shown above does not amplify the mutant DNA efficiently due to the long size of amplicon.