Basic Genotyping Protocol:

(1) Isolate DNA from 1cm tail clip from mice during weaning. Follow DNA isolated from tissue kit instructions.
(2) (Optional) Test DNA concentration using nanodrop or H-stain assay.
(3) Set up and perform PCR (see below).
(4) Store samples at 4C until you are able to run samples on DNA gel.

Ingredient	**Per 1 Rxns**	**Per 21 Rxns**
dH2O	13	273
Fermentas buffer	2	42
dNTPs (10mM)	2	42
FOR (5') (10µM)	1	21
REV (3') (10µM)	1	21
Taq Fermentas	0.1	2.1
Total	19.1	401.1

Add 18.5µl of reaction mixture to 1µl of isolated DNA sample.

Ribotag Reaction:
95 for 2 min
95 for 20 sec
57 for 20 sec
72 for 30 sec
72 for 5 min
30 cycles

Band size:
290bp = Ribotag
260bp = WT
program on PCR machine: Ribotag 56

CRE Reaction:
95 for 2 min
95 for 10 sec
63 for 10 sec
72 for 40 sec
72 for 5 min
25 cycles
program on PCR machine: CRE

Band size:

Run samples on DNA gel.

Ingredient	**Volume**
1X TBE	15ml
40% Acry Bis	5ml
10% APS	150µl
Temed	30µl
Will make 2 gels 1.5mm thick.	

(1) Load 2-10µl of sample per lane depending upon robustness of reaction.
(2) Run samples in 0.5-1X TBE, 140mV, 30-45 minutes.
(3) Utilize 100bp or 1kbp DNA ladder. Load 3µl of ladder per lane.