RT PCR analysis of luciferase expression in **ROSALUC X ROSA26-CreERT²** and **ROSALUC X Alb-CreERT²** mice

RT PCR was performed to analyse luciferase expression in livers of induced or non-induced **ROSALUC X Alb-CreERT²** and **ROSALUC X ROSA26-CreERT²**. Frozen liver or lung tissue samples were homogenised using the FastPrep-24 homogeniser and RNA was extracted using the the RNeasy Mini kit (Qiagen) according to the manufacturer’s instructions. Accordingly, 2µg of RNA was used for cDNA synthesis using the Ready-To-Go-You Prime First Strand Beads kit (Amersham Biosciences). For this purpose, Oligo(dT) primer was employed at a concentration of 300µM and cDNA was synthesized as per the manufacturer’s instructions. cDNA obtained was diluted 1:10 and used for subsequent PCR amplification. Amplification of luciferase in the reverse orientation was performed using primer 1 (5’- GTTCCATCTTCCAGCGGATA-3’) and primer 2 (5’-AGACCCCTAGGAATGCTCGT-3’) to yield a band size of 277 bp. Primer 3(5’-GGAAAAACTCGACGCAAGAAA-3’) and 2 (5’-AGACCCCTAGGAATGCTCGT-3’) yield a band size of 285 bp.

Amplification was done for 30 cycles (95°C 30 sec, 58°C 45 sec, 72°C 90 sec).

Lanes 1-4 represent livers isolated from 4 individual **ROSALUC X Alb-CreERT²** mice respectively with lane 1 and 2 being Tam induced mice and lanes 3 and 4 being non-induced mice. Lanes 5 and 6 represent livers isolated from 2 control single transgenic ROSALUC whereas lanes 7 and 8 are liver samples from 2 control single transgenic ROSAConL respectively. Lanes 9-12 are samples from 2 Tam induced bitransgenic ROSALUC X ROSA26-CreERT² with lanes 9 and 11 being liver samples and lanes 10 and 12 being lung samples. Lanes 13 and 14 represent liver and lung samples respectively, isolated from 1 control single transgenic ROSA26-CreERT² whereas lanes 15 and 16 represent liver and lung samples isolated from 1 control single transgenic Alb-CreERT². Lanes 17-20 are samples from 2 non-induced bitransgenic ROSALUC X ROSA26-CreERT² with lanes 17 and 19 being liver samples and lanes 18 and 20 being lung samples respectively. Lane 21 is the water control.

Age of all the transgenic mice range between 2-5 months and Tam inductions were performed as per stated before.