

Genotyping of IR-heterozygous mice
(genotyping of mice with the Neo cassette inserted into the IR-gene)

Principle Investigator: C.R. Kahn

Mastermix for 10 reactions

H ₂ O	178.75 µl
10x PCR buffer	25 µl
dNTP (2.5 mM)	20 µl
MgCl ₂ (50 mM)	7.5 µl
Primer KAWT1 (20 pM/µl)	2.5 µl
Primer WT5 (20 pM/µl)	2.5 µl
Primer New Neo (20 pM/µl)	2.5 µl
Taq Polymerase	1.25 µl

Vortex, then add 1 µl of DNA template to 24 µl of mastermix.

PCR Program

1 cycle

95 °C for 3 minutes

38 cycles

95 °C for 30 seconds

69.5 °C for 30 seconds

72 °C for 30 seconds

1 cycle

72 °C for 5 minutes

Electrophoresis on 3% agarose gel.

Wild-type mice have a nonspecific band of approximately 275 base pairs.
Heterozygous mice have the non-specific band of 275 base pairs and the Neo-IR band of approximately 750 base pairs.

Primer Sequences

KAWT1: 5' ctg tgc act tcc ctg ctc ac 3'

WT5: 5' ctg agc ttg atg tgc acc cc 3'

New Neo 5' gcc tga ccg ctt cct gct tta c 3'