

## Genotyping of the rWT and INS mice

**Reaction 1** is a multiplex PCR used for verification of the mouse AHR. The wildtype innate mouse AHR will give a product of 369 bp whereas the neomycin cassette incorporated into the AHR of AHR-knockout mice will give a product of 515 bp. Heterozygous mice yield both signals. In mice only expressing the rWT or INS transgenes the mouse AHR is of the knockout type (i.e., this reaction produces a signal at 515 bp).

Primers:

Neo-F	TGAATGAACTGCAGGACGAG
Neo-R	AATATCACGGGTAGCCAACG
MAHR-F	GACACAGAGACCGGCTGAAC
MAHR-R	AGCATGTACCATCCAAACAGC

MAHR-F and MAHR-R amplify 369bp product of wt  
Neo-F and Neo-R amplify 515bp of inserted neomycin cassette

### PCR mix

10x PCR buffer	3ul
MgCl <sub>2</sub> (25mM)	2.7ul
dNTP (25mM each)	0.24ul
Neo-R	0.15ul
Neo-F	0.15ul
MAHR-R	0.15ul
MAHR-F	0.15ul
DNA template	2ul
AmpliTaq Gold (5U/ul)	0.2ul
H <sub>2</sub> O	21.26ul

### PCR cycle

(Lid 99.0°C)

- 1: 94.8°C 2 min
- 2: 94.8°C 35 s
- 3: 62.0°C 35 s
- 4: 72.0°C 40 s → back to 2 for 9 cycles
- 5: 94.8°C 35 s
- 6: 57.0°C 35 s
- 7: 72.0°C 50 s → back to 5 for 24 cycles
- 8: 72.0°C 10 min
- 9: 4.0°C pause

**Reaction 2** will discriminate between the transgenic rWT and INS constructs. The product from rWT is 564 bp in size while that from INS is 593 bp.

Primers:      TG-F            CTCAGCAGGAACGAAAGCAC  
                 TG-R            TTCATCCTGGCCTCGAGC

PCR mix

10x PCR buffer	3ul
MgCl <sub>2</sub> (25mM)	2.7ul
dNTP (25mM each)	0.24ul
TG-F	0.3ul
TG-R	0.3ul
DNA template	2ul
AmpliTaq Gold (5U/ul)	0.2ul
H <sub>2</sub> O	21.26ul

PCR cycle

(Lid 99.0°C)

- 1: 94.0°C 3 min
- 2: 94.0°C 30 s
- 3: 63.0°C 30 s
- 4: 72.0°C 52 s → back to 2 for 5 cycles
- 5: 94.0°C 30 s
- 6: 57.0°C 30 s
- 7: 72.0°C 1 min → back to 5 for 29 cycles
- 8: 72.0°C 10 min
- 9: 4.0°C pause