

## AirNT Genotyping by PCR (designed by Markus Muckenhuber)

Primer	Coordinates (mm10)	Sequences 5' → 3'		Function	Location
MM_Typing_004	In Poly-A cassette	ACTCCAGTCATAGCTGCCCTCT	+	AirNT primer	Box 35 – 100
MM_Typing_006	chr17:12,744,554-12,744,575	GCCCTTTGTAGGTGCCTCTGC	-	Common primer	Box 36 – 2
MM_Typing_015	chr17:12,744,332-12,744,356	ATTCTCTATGGGCCACCCACTTCC	+	WT primer	Box 36 – 8

### Amplicon

AirNT	371bp	(MM_Typing_004 + MM_Typing_006)	(chr17:12,744,358-12,744,575)*
WT	244bp	(MM_Typing_006 + MM_Typing_015)	(chr17:12,744,332-12,744,575)

\* Genomic part of the AirNT band (218bp). The inserted Poly-A cassette (chr17:12,744,357-12,744,358) part (153bp) cannot be found in the mouse genome.

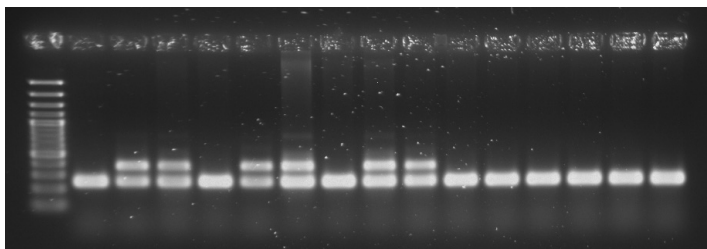
### PolyA cassette sequence

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gacctgagaactcagggtgagttggggacccttgattgtcttnttttcgctattgtaaaatcatgttatatggagggggcaaagtttcagggtgtgttagaatgggaagatgctccttgatcacctggaccctcatgataattgttctt
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ctcttagagatccctcgaggtcgacggtatogataagctcgaggacctaataactctgtagacatacaltatncaaggttatattaagggttatgaatatgacgaattcctgcagcccg
```

PCR Program		
95°C	5'	
95°C	30"	35x
60°C	30"	
72°C	1'	
72°C	5'	
10°C	∞	

### AirNT mastermix calculation

Number of reactions:		32
	per reaction	Mastermix
water	7.90	252.8
5x buffer (GoTaq flex, Promega)	5.00	160
Betaine (5M)	4.00	128
MgCl <sub>2</sub> (25mM)	1.50	48
dNTPs (10mM)	0.50	16
MM_Typing_004 (10µM)	1.00	32
MM_Typing_006 (10µM)	2.00	64
MM_Typing_015 (10µM)	1.00	32
Taq Polymerase (GoTaq G2 flex, Promega)	0.10	3.2
<b>Total</b>	<b>23.00</b>	<b>736.00</b>
Template	2.00	
(ideally 50ng/µl, but significantly lower or higher concentrations may work)		



WT = 244bp, AirT = 371bp (100bp ladder Fermentas)

2% agarose TAE midi-gel (13.5cm (W) x 16cm (L))  
100V x 45min

# AirnT Genotyping by Southern blot

(Barlow lab protocol)

## Probe & enzyme

Enzyme: EcoRI

Probe: Msi

1013bp

(chr17:12,741,515-12,742,527; GRCm38/mm10)

probe F primer

CGCGTTAGAGGATTCCGCAA

probe R primer

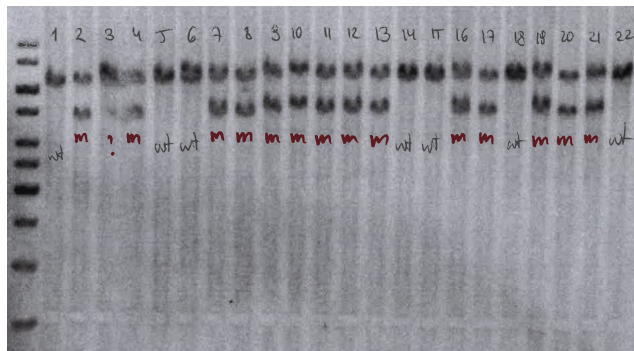
AATCCTCCCCTGTGCAGTTTGC

## Band sizes

WT = 6.3kb

AirnT = 4.7kb

(0.8% gel, 24cm long, 120V x 4hours)



WT = 6.3kb, Mut = 4.7kb