

## Cyb561 (MALD; EPD0037\_3\_G01)

Allele: *Cyb561<sup>tm1a(EUCOMM)Wtsi</sup>*

### Genotyping Information

These mice may be genotyped through a combination of separate PCR reactions that detect *LacZ*, the gene-specific wild type allele, and a mutant allele-specific short range PCR. Interpretation of the consolidated results produces the genotype of the mice.

For example: *LacZ* positive, mutant positive, wild type positive = heterozygous.

#### ➤ PCRs primer pairs and expected size bands

PCR type	Forward primer	Reverse primer	Expected size band (bp)
Mutant PCR	Cyb561_120_F	CAS_R1_Term x	142
Wild type PCR	Cyb561_120_F	Cyb561_120_R	382
LacZ PCR	LacZ_2_small_F	LacZ_2_small_R	108

#### ➤ Primer sequences

Primer name	Primer sequence (5' > 3')
CAS_R1_Term	TCGTGGTATCGTTATGCGCC
Cyb561_120_F	CAGCCCTACCCAGAAAATG
Cyb561_120_R	GTGCTCCATTCTGAGACCCC
LacZ_2_small_F	ATCACGACGCGCTGTATC
LacZ_2_small_R	ACATCGGGCAAATAATATCG

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➤ **Reaction**

Reagent	μl
DNA (~50-100 ng)	1.0
10x Buffer	2.0
MgCl <sub>2</sub> (50 mM)	0.6
PtTaq (Platinum Taq (Invitrogen))	0.2
dNTPs (100 mM)	0.2
Primer 1 (10 μM)	0.4
Primer 2 (10 μM)	0.4
H <sub>2</sub> O	<u>15.2</u>
<b>Total</b>	<b>20.0</b>

➤ **Cycling conditions**

**Wild type and mutant PCRs**

Cycle	Temp	Time
1	94 °C	5 min
2	94 °C	30 sec
3	58 °C	30 sec
4	72 °C	45 sec
5	Go to '2' + 34 cycles	
6	72 °C	5 min
7	12 °C	forever

**LacZ PCR**

Cycle	Temp	Time
1	94 °C	5 min
2	94 °C	30 sec
3	60 °C	30 sec
4	72 °C	30 sec
5	Go to '2' + 34 cycles	
6	72 °C	5 min
7	12 °C	forever

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