

Rufy2 (MDDP; EPD0176_5_C11)

Allele: *Rufy2*^{tm1a(EUCOMM)Wtsi}

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 | Rufy2_50365_F | CAS_R1_Term x | 232 |
| Wild type PCR | Rufy2_50365_F2 | Rufy2_50365_R2 | 323 |
| LacZ PCR | LacZ_2_small_F | LacZ_2_small_R | 108 |

➤ Primer sequences

| Primer name | Primer sequence (5' > 3') |
|----------------|---------------------------|
| CAS_R1_Term | TCGTGGTATCGTTATGCGCC |
| Rufy2_50365_F | CTTCTTACGGCCTCTGTGGG |
| Rufy2_50365_F2 | GGCTGGCCTGGAACCTACTG |
| Rufy2_50365_R2 | CTGATGGAGGCAGGAAAGAG |
| LacZ_2_small_F | ATCACGACGCGCTGTATC |
| LacZ_2_small_R | ACATCGGGCAAATAATATCG |

This technical data sheet and information ("Datasheet") is supplied by Genome Research Limited ("GRL").

Although reasonable care is taken in the preparation of this Datasheet, GRL gives no warranties express or implied for any use of the Datasheet or for the accuracy of the Datasheet. GRL assumes no responsibility or liability for any decisions based upon the Datasheet. Without limiting the foregoing the Datasheet was prepared for mice supplied directly from GRL and where copies of this Datasheet are available from third party repositories or distribution centres ("Third Parties") GRL shall not be liable for any inconsistency between the mouse strain supplied by the Third Party and the Datasheet howsoever arising.

Last updated: 24/05/2012

➤ **Reaction**

| Reagent | μl |
|------------------------------------|-------------|
| DNA (~50-100 ng) | 1.0 |
| 10x Buffer | 2.0 |
| MgCl ₂ (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 ₂ O | <u>15.2</u> |
| Total | 20.0 |

➤ **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 |

This technical data sheet and information ("Datasheet") is supplied by Genome Research Limited ("GRL").

Although reasonable care is taken in the preparation of this Datasheet, GRL gives no warranties express or implied for any use of the Datasheet or for the accuracy of the Datasheet. GRL assumes no responsibility or liability for any decisions based upon the Datasheet. Without limiting the foregoing the Datasheet was prepared for mice supplied directly from GRL and where copies of this Datasheet are available from third party repositories or distribution centres ("Third Parties") GRL shall not be liable for any inconsistency between the mouse strain supplied by the Third Party and the Datasheet howsoever arising.

Last updated: 24/05/2012