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## Dync1i2 (MCZN; EPD0525_1_B10)

## Allele: Dync1i2 ${ }^{\text {tm1a(EUCOMM)Wtsi }}$

## Genotyping Information

These mice may be genotyped through a combination of separate PCR reactions that detect LacZ, the genespecific 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 | Dync1i2_39345_F | CAS_R1_Term x | 147 |
| Wild type PCR | Dync1i2_39345_F | Dync1i2_39345_R | 293 |
| LacZ PCR | LacZ_2_small_F | LacZ_2_small_R | 108 |

## > Primer sequences

| Primer name | Primer sequence (5' $\boldsymbol{>} \mathbf{3}^{\prime}$ ) |
| :--- | :--- |
| CAS_R1_Term | TCGTGGTATCGTTATGCGCC |
| Dync1i2_39345_F | TGGCTAACTTGGGATTTGCC |
| Dync1i2_39345_R | CAGCATTGTGAACTGCCTGC |
| LacZ_2_small_F | ATCACGACGCGCTGTATC |
| LacZ_2_small_R | ACATCGGGCAAATAATATCG |

[^0]Last updated: 09/08/2011

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## > Reaction

| Reagent | $\mu \mathbf{l}$ |
| :--- | ---: |
|  |  |
| DNA $(\sim 50-100 \mathrm{ng})$ | 1.0 |
| $10 \times \mathrm{Buffer}$ | 2.0 |
| $\mathrm{MgCl}_{2}(50 \mathrm{mM})$ | 0.6 |
| $\mathrm{PtTaq}^{\text {(Platinum® }}$ ) Taq (Invitrogen)) | 0.2 |
| dNTPs $(100 \mathrm{mM})$ | 0.2 |
| Primer $1(10 \mu \mathrm{M})$ | 0.4 |
| Primer $2(10 \mu \mathrm{M})$ | 0.4 |
| $\mathrm{H}_{2} 0$ | $\underline{15.2}$ |
| Total | $\mathbf{2 0 . 0}$ |

> Cycling conditions

## LacZ PCR

| Cycle |  |  |
| :--- | :--- | :--- |
| 1 | $94^{\circ} \mathrm{C}$ | 5 min |
| 2 | $94^{\circ} \mathrm{C}$ | 30 sec |
| 3 | $58^{\circ} \mathrm{C}$ | 30 sec |
| 4 | $72^{\circ} \mathrm{C}$ | 45 sec |
| 5 | $\mathrm{Go} \mathrm{to}^{\prime} 2$ ' +34 cycles |  |
| 6 | $72^{\circ} \mathrm{C}$ | 5 min |
| 7 | $12^{\circ} \mathrm{C}$ | forever |

Cycle

| 1 | $94{ }^{\circ} \mathrm{C}$ | 5 min |
| :--- | :--- | :--- |
| 2 | $94^{\circ} \mathrm{C}$ | 30 sec |
| 3 | $60^{\circ} \mathrm{C}$ | 30 sec |
| 4 | $72^{\circ} \mathrm{C}$ | 30 sec |
| 5 | $\mathrm{Go} \mathrm{to}^{\prime} 2^{\prime}+34 \mathrm{cycle}$ |  |
| 6 | $72^{\circ} \mathrm{C}$ | 5 min |
| 7 | $12^{\circ} \mathrm{C}$ | forever |

[^1]Last updated: 09/08/2011


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