Strain: VH7186

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| --- | --- |
| Genotype: | algn-5(hd7175[LoxP + myo-2p::GFP::unc-54 3' UTR + rps-27p::neoR::unc-54 3' UTR + LoxP]) V. |
| Type: | Heterozygous |
| QC Results: | PFHP |
| Deletion: | 1325 bp |
| Flanks: | TCCAAAAAATCAATATCTTCACCATTTTCA - [deletion] - TGGAGCTACAAAATTCGCCGATTTTGAAAA |
| DNA Files: | algn-5\_Deletion.ape, algn-5\_Wildtype.ape |

**Primer location and sequences**

Primer 1-4 coordinates are from the Deletion ApE file. Primer 5 coordinates are from the Wildtype ApE file.

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| --- | --- | --- | --- |
| **No** | **Position** | **Name** | **Sequence (5’ to 3’)** |
| 1 | 1354-1374 | algn-5-1 SV F | AGCATGCTTAGTACCTGCTGC |
| 2 | 2786-2809 | pMYO-2 SEC Insertion Primer 2 | CAGAACAATGAGTACAGTGTGTGC |
| 3 | 6321-6342 | NeoR SEC Insertion Primer 2 | ATCTCCTGTCATCTCACCTTGC |
| 4 | 8837-8858 | algn-5-1 SV R | ACCCTTCGATTTCAGTCCATCG |
| 5 | 2541-2562 | algn-5-1 WT F | CCGACGAAACGGCTGATATAGT |

**PCR products**

|  |  |  |  |
| --- | --- | --- | --- |
| **Product (see figure)** | **Primer** | **Product Size** | **QC Result** |
| {US} - Upstream insertion site | 1+2 | 1456 bp | Pass (Band present) |
| {DS} - Downstream insertion site | 3+4 | 2538 bp | Fail (Band absent) |
| {WoM} – WT PCR on mutant | 5+4 | no product, if WT sequence is deleted | Fail (Band present) |
| {WoM} – WT PCR on wildtype | 5+4 | 2226 bp | Pass (Band present) |

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| --- | --- |
| **PCR Program:**  (1) 94˚C - 30 sec  (2) 94˚C - 30 sec  (3) 60˚C - 30 sec  (4) 72˚C - 3 min  (5) To step (2) x 34 times  (6) 72˚C - 5 min  (7) 4˚C - hold |  |