### Generation of the MonX mutation in B6

Insertion mutagenesis was conducted as described in the B6 mutagenesis protocol. Primers utilized as described below. The mutation was confirmed by genome sequencing.

### **Table 1: Primers Used in This Study**

|  |  |  |  |
| --- | --- | --- | --- |
| Target  | Name | Sequence 5’ to 3’ | Location(accession no.) |
| *monX* | M1 | CTCGAGTTTTTCAGCAAGATTTCTGCGACAGCAACTGACCa | 1720659-1720678(NC\_013853.1) |
|  | M2 | GTATTCACGAACGAAAATCGAAGAGAAGCATCGCGCAACAb | 1720204-1720223(NC\_013853.1) |
|  | M3 | GAAAACAATAAACCCTTGCATAGCACAGGCTGATGGTAATAGACb | 1718925-1718946(NC\_013853.1) |
|  | M4 | AGGAGATCTTCTAGAAAGATAGTGACTGTACCATTGTCGCTAa | 1718491-1718512(NC\_013853.1) |
| Spec | S1 | CGATTTTCGTTCGTGAATAC | 5418–5399(KM009065) |
|  | S2 | TATGCAAGGGTTTATTGTTTTC | 4265–4286(KM009065) |
| pJet 2.1/blunt | Fwd | CGACTCACTATAGGGAGAGCGGC | 310-332(EF694056.1) |
|  | Rev | AAGAACATCGATTTTCCATGGCAG | 405-428(EF694056.1) |

**a** Underlining indicates nucleotides introduced to allow In-Fusion cloning into the pJET2.1/Blunt.

**b** Underlining indicates nucleotides introduced to allow In-Fusion cloning with *aad9* (spectinomycin resistance cassette).