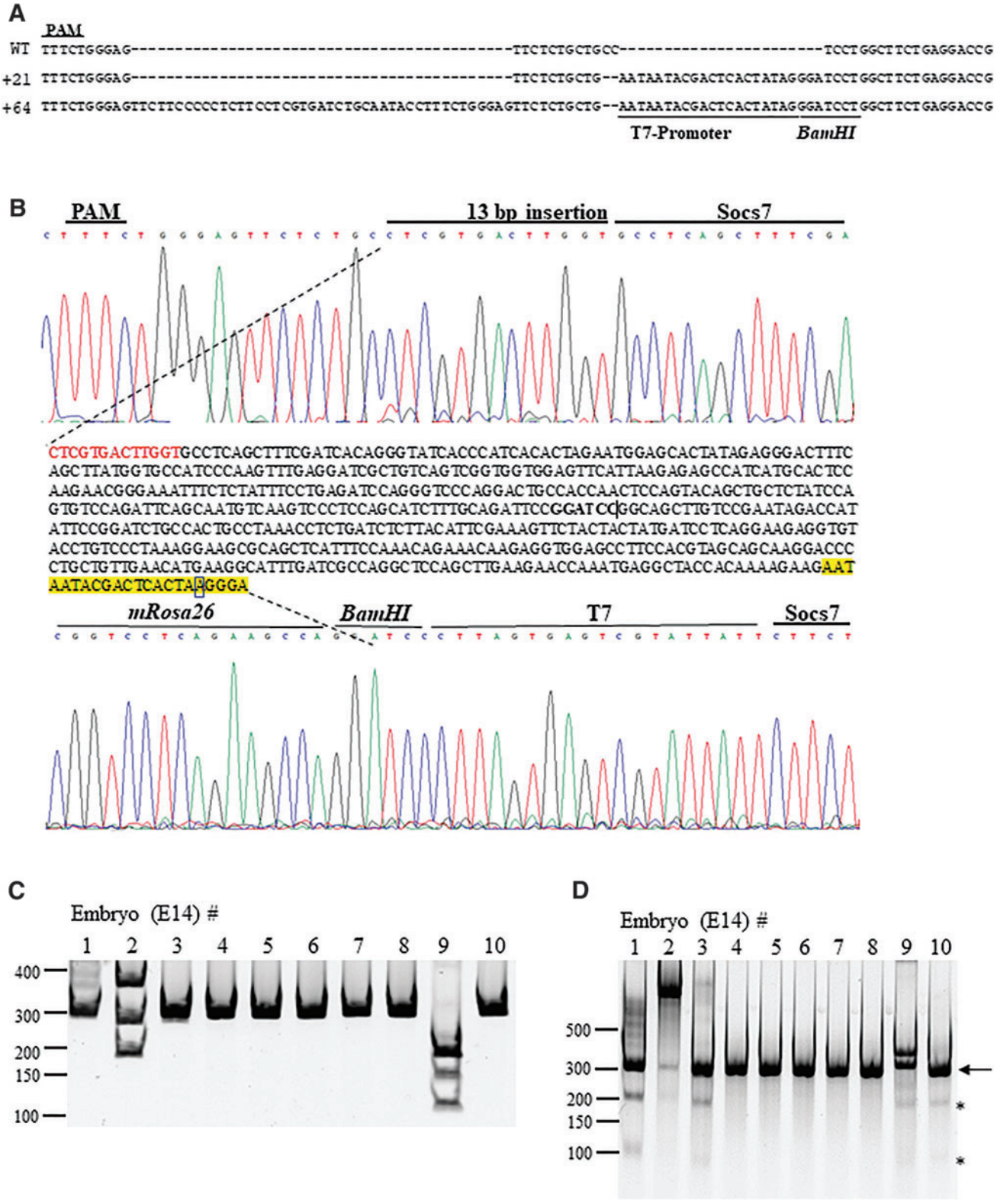
SUPPLEMENTARY FIG. S5. Analysis of E14 embryos from the oligo-donor integration at the mouse *Rosa26* locus. **(A)** Alignment of the WT mouse *Rosa26* sequence with the two most abundant reads (+21 and +64) from the NGS results for E14 embryo #9. Dashes represent missing sequences or gaps between the alignments. **(B)** Sanger sequence data from the large amplification product for E14 embryo #2. Top chromatogram flanks the PAM sequence upstream of the integration site and the bottom chromatogram flanks downstream of the integration site as complementary sequence results. The middle sequence between the chromatograms corresponds to the 13 bp insert (red sequence), spliced Socs7 mRNA (black letters), and the oligo-donor integration of the T7 promoter and *BamHI* restrictions site and extra A base (highlighted sequence; black boxed letter). The location of those corresponding sequences within the chromatogram is underlined above each panel. The bold and boxed letters correspond to the internal *BamHI* site found within the Socs7 sequence. **(C)** *BamHI* restriction digests of the PCR amplicons resolved via 10% acrylamide gels. **(D)** DNA mismatch detection assays using Cel-I enzyme for the oligo-donor E14 embryos. The right arrow represents the expected size of the PCR amplification product (302 bp), and the asterisk marks the predicted fragments for gRNA #1 (201 bp, 101 bp). The numbers to the left of each gel represent the location of the DNA size markers in base pairs.


A
PAM
WT TTTCTGGGAG----------TTCTCTGCTGCC----------TCCTGGCTTCTGAGGACCG
+21 TTTCTGGGAG----------TTCTCTGCTG--AATAATACGACTCACTATAGGGATCCTGGCTTCTGAGGACCG
+64 TTTCTGGGAGTTCTTCCCCCTCTTCCTCGTGATCTGCAATACCTTTCTGGGAGTTCTCTGCTG--AATAATACGACTCACTATAGGGATCCTGGCTTCTGAGGACCG
T7-Promoter
BamHI
B
PAM
13 bp insertion
Socs7
CTCGTGACTTGGTGCCTCAGCTTTCGATCACAGGGTATCACCCATCACACTAGAATGGAGCACTATAGAGGGACTTTC
AGCTTATGGTGCCATCCCAAGTTTGAGGATCGCTGTCAGTCGGTGGTGGAGTTCATTAAGAGAGCCATCATGCACTCC
AAGAACGGGAAATTTCTCTATTTCCTGAGATCCAGGGTCCCAGGACTGCCACCAACTCCAGTACAGCTGCTCTATCCA
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CTGCTGTTGAACATGAAGGCATTTGATCGCCAGGCTCCAGCTTGAAGAACCAAATGAGGCTACCACAAAAGAAGAAT
AATACGACTCACTATAGGGGA
mRosa26
BamHI
T7
Socs7
C
Embryo (E14) #
1 2 3 4 5 6 7 8 9 10
400
300
200
150
100
D
Embryo (E14) #
1 2 3 4 5 6 7 8 9 10
500
300
200
150
100
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